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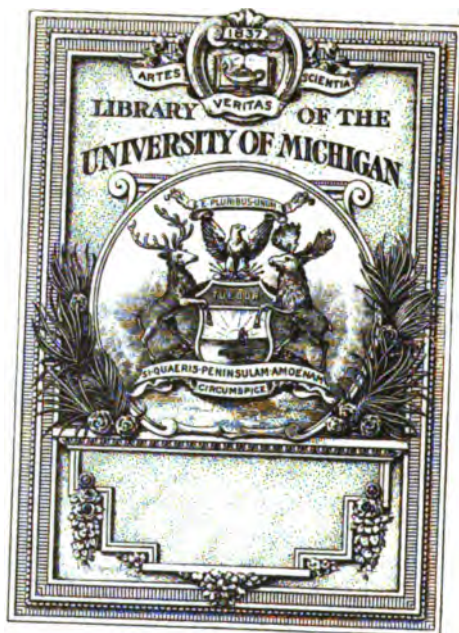
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A MANUAL
OF
CLINICAL LABORATORY
METHODS

BY
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To My Wife
ANNIE GLEDHILL NICHOLS

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PREFACE.

IT is the purpose of this work to present in practical and systematic form the most important laboratory methods employed in clinical medicine. Covering established methods, it cannot profess to present much that is new or original, or to cover a field not already occupied, but must base its *raison d'être* on the collection of numerous technical procedures within small compass and on presentation of the subject in a manner convenient and clear to students and practitioners of human medicine, to whom it is hoped the work may prove of use.

JOHN BENJAMIN NICHOLS.

WASHINGTON, D. C., December, 1901.

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A MANUAL OF CLINICAL LABORATORY METHODS.

I. INTRODUCTION.

IN the clinical practice of medicine there are two distinct and equally important divisions: diagnosis, the determination of the nature of the disease condition present, and therapeutics, the treatment of that condition. The processes and nature of disease are often so obscure, and their recognition is so essential as a guide to proper treatment, that the clinician needs to utilize all information that may throw light on his cases. Various classes of data are available for diagnostic purposes, such as the history of the case, the subjective phenomena experienced and communicable by the patient, and the objective characteristics exhibited by the patient and determinable by various methods of investigation. In the latter class of data are included the results derived by examination of fluid and solid materials obtainable from the patient's person.

Such materials it is the province of the clinical laboratory to elaborate. The chemical, microscopical, bacteriological, and other methods utilized in this kind of work are very diverse in their nature, principles, and technique; yet as all these methods have to be applied to the examination of the same material, and the assemblage of the requisite reagents and apparatus naturally results in the equipment of a laboratory convenient for working purposes, it comes about that the worker in the clinical laboratory combines the functions of the chemist, the microscopist, and the biologist, and this entire field forms quite a distinct department of medical work.

The field of the clinical laboratory is, then, the investigation by appropriate methods (chiefly chemical, microscopical, and bacteriological) of such fluid and solid materials, secretions,

ejecta, tissues, and the like, as are obtainable from the diseased subject, for the purpose of obtaining whatever data may aid in ascertaining the nature and course of the disease processes present. It is the object of this treatise to present, for the use of the student and worker, the most important practical clinical laboratory methods at present in general use, together with a sufficient exposition of the principles on which they are based, to enable them to be intelligently and rationally understood and their results to receive the proper clinical interpretations. In the following pages, in each branch of the subject the composition and general characters of the material to be examined are first considered, so as to place the technique on a rational basis and permit clinical interpretation to be made of the conditions and variations found in normal and abnormal circumstances; secondly, the technique and methods of examination are given by which the various facts of diagnostic usefulness are elicited.

The practical usefulness of the results obtained by clinical laboratory methods should be estimated at their proper value, and neither overestimated nor underrated. In many cases the laboratory findings are essential to the making of the diagnosis. In other cases they furnish valuable or even pathognomonic data, which yet may not be essential to the making of a diagnosis with reasonable certainty. In many cases the information elicited may be of no positive or definite significance, or significant only in a negative way. On the one hand, laboratory work should not be regarded with excessive enthusiasm, or with the expectation of pathognomonic results in every case, or to be an invariably short, easy, and sure road to diagnosis. On the other hand, these methods should not be underrated as merely academic and impractical in character; nor does the laboratory worker desire to be regarded and treated as a visionary enthusiast who enjoys spending his time and labor, regardless of other compensation, in examining specimens patronizingly handed him. Properly utilized the field of usefulness of clinical laboratory methods is very large, but they must not be expected to take the place of other diagnostic aids. The methods are largely of recent development, and the field is still growing both in technical procedures and clinical usefulness; a well-equipped clinical laboratory is now an essential adjunct to every modern hospital.

Clinical laboratory findings once obtained must be properly

interpreted in order to utilize them advantageously and to avoid erroneous conclusions. Negative results from single or a few examinations do not necessarily disprove the existence of the disease in question; thus, failure to find tubercle bacilli, the malarial parasite, or the Widal reaction does not necessarily prove the absence of tuberculosis, malaria, or typhoid fever. Only repeated negative results in such cases under favorable conditions can be safely given a negative interpretation. Positive findings are much more definite in their significance. The results should be taken in their clinical connections, and all data obtainable, subjective symptoms and results of physical examination, as well as microscopical and chemical findings, should be considered together in making diagnosis. Laboratory work is an essential part of clinical medicine, precisely corresponding to physical examination, and should be given its place, no more and no less, in the clinical whole.

The materials available for examination by clinical laboratory methods during life and in time for useful therapeutic indications are limited to the fluid and solid substances practically obtainable, including most of the secretions and excretions of the body, ejecta, pathological fluids, and small portions of solid tissues. Some of these materials are obtainable with ease and in ample quantity, as urine, fæces, sputum; some are easily obtainable, but only in small amount, as blood; some are obtainable in sufficient quantity but with considerable difficulty or discomfort, as cerebro-spinal fluid, stomach contents, portions of tissues. In addition to the substances obtainable during life in time for therapeutic usefulness, after death and to a certain extent after operations any of the materials of the body are available for examination for the retrospective light which may be thrown on the case.

Histological technique, or the examination of solid tissues, constitutes an important part of clinical laboratory work. It alone is an extensive subject, and from lack of space its consideration has necessarily been omitted here.

II. LABORATORY EQUIPMENT AND GENERAL METHODS.

The equipment of the clinical laboratory must be conditioned on the available resources as to space and funds. If necessary, very satisfactory work can be done within small space. For microscopical purposes a clear northern exposure, unobstructed by trees, buildings, or other objects, is desirable. Working-tables, shelf room, drawers, sinks, and water and gas supply should be provided. A variety of apparatus, reagents, and supplies is necessary. Among the most important items are the microscope, microtome, freezing apparatus, centrifuge, bacterial incubator, hot-air and steam sterilizers, delicate scales. It is not necessarily the most expensive and most complicated apparatus that is the most desirable. With ordinary ingenuity much useful apparatus can be readily improvised.

The microscope should be of the highest grade, provided with iris diaphragm, Abbe condenser, triple nosepiece, two oculars, objectives of about 2 centimetres, 4 or 5 millimetres, and 2 millimetres (oil-immersion) focal length. A mechanical stage and micrometer outfit are very desirable adjuncts.

Cleanliness, order, and system in managing the laboratory are desirable for the sake of appearance and to promote and facilitate the work. Blank forms are neat and useful to guide and facilitate the examinations, and valuable records may be kept with little labor.

Reagents.—Much often depends on the reagents used being exactly right. This is especially the case with aniline stains, minute variations in whose composition or mode of manufacture often make all the difference between success and failure. One commercial make often produces results that another of the same substance does not yield. Hence in some formulæ it is necessary to specify the maker's name to help insure the desired result. For many purposes, for instance, the stains of Grübler, of Berlin, are the most reliable and satisfactory. Chemically

pure substances and accurately prepared volumetric solutions should be handled with the same scrupulous care to avoid contamination or alteration that is exercised with pure bacterial cultures.

In preparing accurate solutions of many chemicals the water of crystallization must be taken into consideration. Perfect and well-formed crystals, dry, are the only uniform standard for direct weighing; in such crystals as contain a definite proportion of water of crystallization the weight of the active principle is only a part of the full weight of the crystals. In calculating formulæ for solutions this factor is taken into account. Substances that deliquesce or effloresce, that is, absorb water or give it off (in either case losing their crystalline form), can not be weighed with any pretence to accuracy, since the amount of water present is an unknown and indefinite factor. Exact volumetric solutions of such substances (sodium hydrate, for instance) must be prepared by standardization with solutions of other substances that can be more directly made.

Cover-glasses.—Microscopical cover-glasses are sold in four thicknesses, Nos. 0, 1, 2, and 3. Of these No. 1, between .12 and .17 millimetre in thickness, is thin enough for oil-immersion lenses, and is the best for general purposes. No. 2 can be used where the oil-immersion lens is not required. No. 0 is too fragile for use and of no advantage over No. 1. Sizes of 18 to 21 millimetres, square or circular, are the most convenient; the squares are cheapest.

Cleaning Glassware.—It is of the greatest importance that the glassware used in the laboratory, especially cover-glasses and slides for microscopical purposes, should be absolutely clean. There is no better cleaning fluid for glass than the following:

Potassium bichromate.....	10
Sulphuric acid, commercial.....	10
Water.....	100

The glassware, just purchased or soiled by use, is left in this fluid for twenty-four hours, or for an indefinite time, and then thoroughly rinsed with water. Slides and cover-glasses should then be kept in alcohol, in large-mouthed jars; as needed for use they are dried with clean gauze.

Extemporaneous microscopical work is largely required in the

laboratory. Small solid masses may be teased in a watch-glass or on the slide, or may be crushed and spread out between the cover-glass and slide, until they are thin or divided enough for microscopical examination. Decinormal (0.6 per cent) sodium-chloride solution is generally used as a medium for such manipulations and for temporarily mounting the specimens for examination; water or glycerin may also be used. In examining clear colorless unstained specimens in this way the substage illumination should be dimmed to the point of the best effect by contracting the iris diaphragm or lowering the condenser.

Granular material may be rubbed up with a suitable fluid so as to dilute and scatter the granules sufficiently. Fluids are examined by placing a drop or two on the slide and covering with a cover-glass. To preserve fluid mounts for a few hours a rim of vaseline may, with a camel's-hair brush, be applied around the dried edges of the cover-glass so as to prevent evaporation.

Undissolved solid elements or particles suspended in liquids, if not sufficiently concentrated, may be collected by allowing them to subside as a sediment after standing a long enough time, or by throwing them down with the centrifuge. When thus concentrated, one end of a glass tube with the other end closed by the finger is passed to the sediment in the bottom of the liquid, which then on removal of the finger above flows up into the tube; on replacing the finger the material within the tube can be removed and deposited on a slide for examination. The amount of sediment allowed to flow into the tube can be readily controlled by the finger above, the finger and end of the tube being dry.

Micro-chemical tests can be made by adding reagents to the material under examination and watching the reaction under the microscope. A drop of each of the materials used may be mixed on the slide and the cover then applied; or with the material being examined already under the cover-glass a drop of the reagent is placed at the edge of the cover-glass and allowed to flow under. In this manner starch granules may be demonstrated by the addition of iodine solution, fat brought out by Sudan III., acetic acid added to clear up cell granules, etc.

A common method of extemporaneous microscopical examination is by means of **cover-glass preparations**. These are prepared by smearing or spreading a small portion of the material

(as pus, etc.) over the surface of a cover-glass with a platinum loop, or otherwise, and allowing it to dry in a thin film; or a drop of the material may be placed on one cover-glass, another glass immediately placed over it, and the material pressed out or allowed to spread out in a thin layer between the two cover-glasses; the glasses are then slid (not lifted) apart, leaving a thin film on one side of each cover-glass. When dried, such cover-glass preparations may be stained at once, or kept indefinitely before further treatment. The next step after drying the films is *fixation*; not, as many novices think, to make the film adhere to the glass, but to coagulate the albuminous principles so that the cells will stain well. Various methods of fixation are employed for different purposes. Fixation is most frequently done by "flaming" the cover-glass preparations, that is, by grasping them with forceps by one edge and then passing them three or four times at a moderate speed and at short intervals through the flame of a burner. Specimens may also be fixed with alcohol, alcohol and ether, or in numerous other ways. After fixing they are stained by appropriate methods. The whole procedure requires only a few minutes for execution.

Preservation of Specimens.—For preserving specimens of organs and tissues, for exhibition purposes or storage, a number of preservative fluids may be used. Weak solutions of formaldehyde, 1 or 2 per cent (= 2 to 5 per cent of formalin), are cheap, effective, and extensively used. Alcohol of about 70 per cent strength is an excellent preservative medium; strong alcohol makes the specimens too hard, an excess of water macerates them. Other fluids are used for special purposes. At first the fluid should be changed frequently until it remains free from blood or turbidity. The specimens should not be too crowded in the fluid.

Kaiserling's method of preserving gross specimens for exhibition purposes has the advantage of retaining the natural color of the tissues. The method is as follows:

1. Fix for one to five days according to size, in the dark, in the following:

Potassium nitrate.....	15
Potassium acetate.....	80
Formalin (40 per cent formaldehyde)...	200
Water.....	1,000

2. Place in 80 per cent alcohol one to six hours, then in 95 per cent alcohol one or two hours.

3. Preserve, in the dark, in

Potassium acetate	200 grams.
Glycerin.....	400 c.c.
Water.....	2,000 c.c.

Weights and Measures.—The metric system is used throughout in this work. The equivalents between the Apothecaries' or Troy and the metric system are as follows:

$$1 \text{ kilogram} = \left\{ \begin{array}{l} 15,433.6 \text{ grains} \\ 2.679 \text{ pounds Troy} \\ 2.205 \text{ pounds avoirdupois} \end{array} \right\} = \text{approxi-} \left\{ \begin{array}{l} 2\frac{1}{2} \text{ pounds Troy} \\ 2\frac{1}{2} \text{ pounds avoirdupois} \end{array} \right.$$

1 gram = exactly 15.4336 grains, or approximately $\frac{1}{4}$ drachm.

1 centigram = " .1543 " " " $\frac{1}{4}$ grain.

1 milligram = " .0154 " " " $\frac{1}{16}$ grain.

1 ounce Troy = exactly 31.1009 grams, or approximately 31 grams.

1 drachm = " 3.8876 " " " $\frac{1}{4}$ ".

1 grain = " .0648 " " " 6 centigrams.

1 litre = exactly 33.8662 fluid ounces, or 2.1168 pints.

1 millilitre or cubic centimetre } = " 16.2567 minims.

1 pint = exactly 473.4736 cubic centimetres.

1 fluid ounce = " 29.5296 " " "

1 minim = " .0615 " " "

1 metre = exactly 39.37 inches, or approximately 40 inches.

1 centimetre = " .3937 " " " $\frac{1}{2}$ inch.

1 millimetre = " .0394 " " " $\frac{1}{25}$ ".

1 micromillimetre (μ) = " .000039 " " " $\frac{1}{25,000}$ "

1 inch = exactly .0254 metre, or approximately 25 millimetres.

Degrees Centigrade (C) may be converted into degrees Fahrenheit (F) and *vice versa* by the following formulæ:

$$C = \frac{5(F - 32)}{9}.$$

$$F = \frac{9C}{5} + 32.$$

The **specific gravity** of organic fluids is ordinarily taken by means of special floating instruments on the order of a hydrometer. For more accurate determinations, or where only a small amount of liquid is available, insufficient to float the instrument, the pycnometric method of direct weighing may be employed. This may be carried out by the use of receptacles in which a

known volume of fluid can be very accurately measured; or the necessary receptacle may be prepared impromptu. In the latter case a piece of glass tubing of proper capacity is drawn to a fine (but permeable) point at each end. This tube is accurately weighed while empty and dry; it is then completely filled with the fluid under examination, the outside dried, and weighed; it is then similarly filled with pure water and again weighed. In this manner the weights of an equal volume of water and the fluid tested are obtained, from which the specific gravity of the latter can be calculated by dividing its weight by that of the water.

Ethereal Extracts.—Reference will be occasionally made in this work to the extraction of substances from their solution in water by means of ether, chloroform, or other fluid not miscible with water. This is done by shaking the watery solution with ether or chloroform, etc.; the latter will extract or take into solution certain substances from the water, and after standing the two fluids will separate. Either the water or the other fluid may then be removed separately with a pipette, and the desired substance thus separated; or by placing it in a filter paper previously saturated with either water or chloroform (for instance), the watery or chloroform portion respectively will filter through alone.

Centrifugal Apparatus.—The centrifugal machine (Fig. 1) is a very great convenience in laboratory work, and for some purposes is a necessity. Its purpose is to concentrate the undissolved solid materials in any liquid (as blood, urine, etc.), in the bottom of the liquid, either with a view of determining the relative amount or bulk of the solid constituents (as in "centrifugal analysis") or of collecting them for microscopical examination. This is accomplished by the fluid being placed in tubes



FIG. 1.—Hand Centrifuge, showing urine tubes attached, hæmatokrit or blood tubes above. (Bausch & Lomb.)

at the ends of a horizontal arm, which is revolved at a high rate of speed by means of hand, electric, or water power acting on a gearing. There are separate attachments for blood, urine, sputum, milk, etc. The powerful centrifugal action forces the heavier undissolved solids very quickly and compactly to the bottom of the fluid.

Contact Tests.—Testing by the contact method is frequently practised, as in examining for albumin, hæmoglobin, etc. The method consists in introducing the test fluid and the fluid to be tested together into a test tube or conical glass so that instead of mixing they form separate layers, the lighter fluid overlying the heavier. The reaction takes place at or near the plane of junction of the two fluids. Either fluid may be introduced first, preferably the heavier; the tube or glass is inclined so that its side forms a slight slope, and the other fluid is then allowed to flow from a pipette slowly and gently down the inclined side so as to overlie or underlie the other fluid, without being mixed; the glass is then carefully turned upright again. Or the lighter fluid may be first introduced; the other fluid is drawn into a long pipette; the upper end of the pipette being tightly closed with the finger, the point of the pipette is passed to the bottom of the glass, and the finger carefully raised so as to permit the fluid to flow gently from the pipette and underlie the other fluid.

For observing delicate color changes in contact or other tests, the test tube is best held in front of a white background, as a sheet of paper; the light from the window should fall upon the white background, whence it is reflected through the test tube to the eye of the observer. For observing white precipitates or clouds, as albumin, an unilluminated black background is preferable.

Before taking up the special lines of laboratory investigation in detail it will be convenient to consider a few methods and tests of general application.

Fat.—The presence of microscopic particles of fat is best determined by treatment with Sudan III. The test solution is prepared by first making a saturated solution of Sudan III. in alcohol; after standing several days one part of this solution is mixed with one part of alcohol and one part of water; the mixture is at first turbid, but clears on standing. Sudan III. has an affinity for fat only, staining fat particles red and leaving everything

else uncolored; the specimens must not be treated with alcohol either before or after staining, as that dissolves out the fat and stain. Permanent mounts must be made in glycerin-jelly, or similar medium. For making the test a drop or two of the Sudan III. solution is added to a few drops of the fluid or material (as gastric fluid, fæces) to be tested for fat, or is allowed to flow under the cover-glass covering the latter; particles of neutral fat are by this procedure conspicuously differentiated under the microscope by their taking a red color. Sections of solid tissues, cut by the freezing method and untreated by alcohol, are stained in the solution and examined or mounted in a watery or glycerin medium.

Starch in solution or granular form is tested for by free iodine, with which it strikes a deep-blue color. The stock test solution (Lugol's, Gram's) consists of iodine 1, potassium iodide 2, in a variable amount of water, say 300 parts. For microscopical purposes a drop of this may be mixed with a drop or two of the sediment or fluid under examination, or allowed to flow under the cover-glass; starch granules, if present, are stained deep-blue. For testing liquids for the presence of starch or erythro-dextrin dilute the iodine solution with water till it is of a light-yellow color, and add a few drops of the suspected fluid; if a blue color develops *starch* is present; if a deep-brown color appears *erythrodextrin* is present. *Achroödextrin* gives no reaction.

Glycogen granules when treated with the above iodine solution turn a deep mahogany-brown, and may be thus differentiated under the microscope.

Iodides.—Occasions at times arise in clinical work to test the secretions (saliva or urine) for the presence of iodides or other iodine combinations experimentally or otherwise administered. The starch method is employed for this purpose; starch is not affected by iodine in combination, but on setting the iodine free with nitric acid or chlorine the characteristic blue color develops. The test is best applied as follows: A watery solution or thin paste of starch is made with the aid of heat; filter paper is saturated with it, dried, and cut into pieces of suitable size. These pieces keep until ready for use. To make the test one of the pieces is well moistened with the fluid to be tested, and then touched with a drop of nitric acid. The appearance of a blue color indicates the presence of an iodine compound.

Occasions constantly arise in laboratory practice where unusual or unexpected results, obscure reactions, or perplexing conditions are presented. Methods could hardly be presented that would obviate all difficulties that might possibly arise; these must be met and mastered at the time by the exercise of common sense, judgment, and ingenuity, guided by enlightened experience.

III. THE BLOOD.

The blood is so intimately concerned in the processes of life and disease, and comes into such close contact with all parts of the body, that the conditions manifested by it throw important light on the nature of many vital and abnormal processes that may be in progress, and the clinical examination of the blood affords assistance in diagnosis in many conditions, both in a positive and negative way. Specimens of blood are easily obtainable for purposes of examination, though only in minute quantities.

A. CONSTITUTION OF THE BLOOD.

The blood is composed of a fluid portion, the plasma, in which are suspended large numbers of cells or corpuscles, which are of three kinds, red blood corpuscles, leucocytes, and blood plates.

The total quantity of blood in the body (normally about one-thirteenth of the body weight) is doubtless variable, and would be a valuable clinical datum if there were any method of determining it. The relative proportions of the plasma and corpuscular elements of the blood are easily determinable; but to ascertain if there is any absolute total increase or decrease in the plasma or hæmocytes, and its extent, which is the real criterion of an anæmia or plethora, it would be necessary to know also the total volume of blood.

The normal color of arterial blood is bright red, that of venous blood darker; in abnormal conditions the color may vary, being paler in anæmia and hydræmia, darker in concentrated blood, cherry-red in carbon-monoxide poisoning, dark and venous in poisoning by hydrocyanic acid and other poisons, somewhat milky in leukæmia. The consistency of blood is normally somewhat creamy, but may be thinner and watery in anæmic and hydræmic conditions, thicker and less fluid where the blood is more concentrated (as cholera). The reaction of the blood is

normally alkaline, but the degree of alkalinity may vary, and the reaction may even be acid (in cholera Asiatica).

The **specific gravity** of the blood is normally about 1.056 to 1.060, somewhat higher in males than in females. In conditions in which the proportion of solids is reduced, as in anæmia, it may range down to 1.030 or lower; the specific gravity should be a useful index of the degree of concentration or dilution of the blood. To a large extent the specific gravity varies parallel with the hæmoglobin.

Blood Plasma.

This is a clear, homogeneous, colorless fluid normally comprising about three-fifths of the volume of the entire blood. Its quantity is susceptible of variation in different circumstances. It may be *increased* in amount from ingestion of fluids, subcutaneous or intravenous injection of fluid, in cedematous conditions, or as a result of lowered blood pressure or vascular dilatation, which permits the entrance of lymph from the lymphatic vessels; this condition, known as "hydræmia," causes a *dilution* or thinning of the blood, the corpuscles not being correspondingly increased, and hence comprising a smaller relative proportion of the whole amount of blood.

The amount of the plasma may be *diminished*, after profuse loss of fluid from free purging, vomiting, or sweating, or from insufficient ingestion of water; the corpuscles not being correspondingly reduced, the result of such a diminution of the fluids of the plasma is a *concentration* of the blood and relative increase in the proportion of the corpuscles; such losses of serum from the blood are very quickly replaced. Increase or decrease in the volume of the plasma (the corpuscles not being correspondingly affected) is manifested in hæmatologic examination by a relative contrary change (decrease or increase respectively) in the number of corpuscles per cubic millimetre; and it is necessary to take other conditions into account in order to determine whether abnormalities in the corpuscle-count are due to changes in the amount of the plasma or of the corpuscles.

The blood plasma consists of water holding in solution normally about 9 per cent of various substances, as albumins, globulins, fibrin factors, chlorides, phosphates, compounds of potassium, sodium, and calcium, glucose, glycogen, fat, fatty acids,

urea, uric acid, and other substances, besides adventitious material derived from food and other ingested substances. Variations in the kind and amounts of the various constituents of the plasma occur according to the substances absorbed, the processes of metabolism, or the action of the excretory organs; thus there may be a decrease in the amount of proteids, an increase or decrease of the fibrin factors, an increase of glucose (diabetes mellitus), an increase of katabolic products or leucomains (as in uræmia, lithæmia), or increase of fat (lipæmia); or unusual and abnormal substances may be present, as bile constituents (cholæmia), toxic agents of extra- or intra-corporeal origin (toxæmia), bacterial toxins, antitoxins, and agglutinins developed in consequence of infectious conditions. Qualitative and quantitative determinations of many of the chemical constituents of the blood plasma would afford valuable clinical information in many cases; but unfortunately, owing to the limited quantities of blood ordinarily available for examination and the difficulty of identifying many of the substances (as toxins), practicable methods of investigating the blood plasma have as yet been developed only to a very limited degree. There are a few tests, as for glucose (Bremer's, Williamson's), glycogen (iodine), agglutination tests, that require only minute quantities of blood; and other constituents can be estimated if sufficient amounts of blood can be obtained. But there are many substances, especially those having toxic action, the recognition of which, if it were practicable, would be of great aid in diagnosis and treatment.

One important class of substances, the **agglutinins**, are amenable to examination. The agglutinins are substances which appear in the blood serum in consequence of bacterial infection, various bacteria producing their own specific agglutinins. These substances when added to cultures of the corresponding germs cause the bacteria to become aggregated or "agglutinated" together in clumps or masses, at the same time arresting motility of the bacteria. Each agglutinin affects only its own specific bacterium. The presence of an agglutinin is tested by adding a small amount of blood serum from the case to a culture of the suspected bacteria, under proper conditions; if the agglutinating action occurs, the presence of the agglutinin of that germ is demonstrated, and this again shows that the subject furnishing the serum had suffered infection by the corresponding parasite.

This method is utilized in the diagnosis of typhoid fever, under the common name of "Widal reaction," which is typical of the whole group of agglutination tests. Although chiefly used in typhoid fever, the method is more or less applicable in a few other infectious conditions, as cholera, bubonic plague, Malta fever.

Small particles of fat occasionally appear in the plasma, as after a fatty diet. **Pigment** is sometimes present, notably black pigment granules in malaria, in melanosis, and sometimes, it is said, in Addison's disease ("melanæmia"). The **hæmoglobin** of the red corpuscles sometimes (in toxic or other conditions) passes into solution in the blood plasma ("hæmoglobinaemia"); the serum which separates after clotting is in such cases red instead of straw-colored. **Glycogen** in granules may occur in the plasma normally; it is increased in suppurative conditions, pneumonia, leukæmia, and other conditions, not only in the plasma but abundant in the polynuclear leucocytes.

Granules of a peculiar kind, known as hæmokonia or "blood dust" (Müller), are frequently to be seen in blood plasma. They are very minute in size (1 or 2 micromillimetres in diameter), rounded or irregular in shape, colorless and refractile, and in constant active oscillating or Brownian movement. Their nature is not fully determined, but they are probably minute bits of protoplasm or granules extruded from leucocytes. They occur in both normal and abnormal conditions; ordinarily they are few in number, occurring singly here and there, but sometimes they are present in large numbers. They have no known clinical significance.

Coagulation and Fibrin Formation.—Human blood normally coagulates in about from three to ten minutes. Departures from the normal time of coagulation may occur in consequence of individual peculiarities or pathological processes. In some individuals coagulation occurs almost instantly. In pernicious anæmia, other anæmic conditions, inflammations, and hæmophilia, coagulation is retarded.

The process of fibrin formation may be observed in microscopical preparations of fresh blood, and some judgment as to coagulation or the amount of fibrin may be formed in that way. Under such circumstances numerous delicate, colorless, straight filaments of fibrin appear in the plasma, running in different di-

rections, radiating from points or centres, or interlacing to form a network; blood plates or granular materials are often located at the points of intersection. In general the amount of fibrin formation is increased in infections and inflammatory conditions, running largely parallel with the number of leucocytes and the temperature; it is not increased in the leucocytoses of leukæmia and malignant disease, and is diminished in pernicious anæmia.

In the days of bleeding the character of the blood clot was a matter of clinical significance, but at the present time opportunity for taking advantage of such data is only rarely afforded. The firmness of the clot, the occurrence of the "buffy coat" (the pale upper layer caused by subsidence of the corpuscles when coagulation was slow, or the corpuscles were relatively heavy), or the formation of a concave or "cupped" upper surface of the clot, are characters that were once considered significant, but are now obsolete.

Red Blood Corpuscles.

Red blood corpuscles or erythrocytes consist of a soft, pliable, and elastic proteid stroma, in which hæmoglobin is suspended in solution. Their form and consistency are due to the stroma, their color to the hæmoglobin.

The normal number of red corpuscles in given volumes of blood under ordinary conditions is quite constant, being in adult males about 5,000,000 and in adult females about 4,500,000 in each cubic millimetre of blood. Under various conditions, normal and abnormal, the number varies within wide limits (the extremes observed being 143,000 and about 9,000,000 per cubic millimetre), and these deviations constitute important facts for clinical consideration.

The number of erythrocytes is increased ("polycythæmia") in various conditions. In vigorous health the number may run up to 6,000,000 or even more. For a few days after birth, the number is high, up even to 8,000,000. With increase of altitude above the sea level there is a remarkable increase in the number of red cells, up to 8,000,000 at the altitude of 4,400 metres; the relation of the total volume of the erythrocytes to their number under these circumstances has not been worked out, and the cause of this altitude polycythæmia is very obscure. After

recovering from anæmic conditions increased hæmopoietic activity may cause a marked increase in the number of red cells over the normal. Concentration of the blood by decrease of the plasma, as by loss of fluid from severe sweating, diarrhœa, or vomiting, or by vascular contraction and increased blood pressure causing an expulsion of blood serum into the lymph channels, increases the number of red corpuscles to the cubic millimetre up to 6,000,000 or more. In acute poisoning by phosphorus or carbon monoxide there may be a great increase in the number of red cells, up to 8,000,000 or over. In localities where there is congestion, stasis, or accumulation of corpuscles there may be a local increase of red cells. There may occasionally be conditions of general cellular plethora or stasis exhibiting polycythæmia.

The number of erythrocytes is decreased ("oligocythæmia") especially in anæmic conditions of various origin, primary and secondary, most notably in pernicious anæmia, in which the corpuscles often run as low as 400,000 to 800,000. Diluted or hydræmic conditions of the blood caused by access of fluid from ingestion by the mouth or subcutaneous or intravenous injections, oedematous conditions, decreased blood pressure or vascular dilatation (as after administration of amyl nitrite), permitting entrance of lymph into the blood, lower the count of red corpuscles.

In itself the number of red corpuscles per cubic millimetre simply expresses the quantitative relation between these cells and the plasma, and when the number present is abnormal all the concomitant circumstances must (in the absence of a method of determining the total amount of blood) be taken into account in order to draw a conclusion as to whether the variation is due to changes in the total amount of the corpuscles or of the plasma. Thus in a case of anæmia, a severe sweat or diarrhœa may cause such a loss of fluid as to carry the number of red cells up to or above the normal; and yet the increase would be only apparent and relative, and not real and absolute.

The normal volume of red corpuscles relatively to the total volume of the blood is about 40 to 43 per cent; the ordinary centrifugal method of determining the volume of the corpuscles gives a slightly higher proportion, about 50 per cent. The volume ratio varies in different conditions, depending on (a) the number

and (b) the size of the individual corpuscles. If the size of the corpuscles were constant, the volume would vary in direct ratio with the number of cells; and from a determination of the volume the number could be directly calculated, and vice versa. Variations in the size of the cells in different cases would, however, affect the total relative volume of the corpuscles, though to a less degree than deviations in the number. Independent estimations of both the volume and the number afford a means of calculating the average bulk of the individual corpuscles, or the "volume index." The average volume of the red corpuscles, as calculated from their dimensions, is normally about 85 cubic micromillimetres each; as calculated from the findings given by the centrifugal apparatus the volume is about 100 cubic micromillimetres, which, while somewhat in excess of the real bulk, is the only practicable method of determination and provides a useful standard figure.

The distribution of the red corpuscles is ordinarily probably uniform throughout the circulatory system, so that a specimen taken from the peripheral blood-vessels in the ordinary manner must be regarded as sufficiently typical and representative of the entire blood. In situations where there is a local congestion or œdema, however, there may be a concentration or dilution of the blood, so that specimens from such localities would not be representative of the general blood mass.

The color of the red corpuscles when seen singly is a peculiar pale greenish-yellow, lighter and paler in shade at the centre, where the corpuscle is thinnest, and gradually deepening toward the periphery. The color is due to hæmoglobin, which makes up about 95 per cent of the solid matter of erythrocytes; and variations in the proportionate amount of this important substance cause corresponding variations in the color of the blood as a whole and of the individual corpuscles. The amount of hæmoglobin necessarily to a certain extent corresponds to variations in the number of red corpuscles; but the hæmoglobin may and does vary independently of the number of the cells, disturbing influences usually causing the hæmoglobin to be less in amount than the number of corpuscles would account for. Thus, in anæmic conditions the hæmoglobin is reduced in amount along with the number of erythrocytes; but it is usually reduced relatively more, notably in chlorosis. Great decrease in hæmoglo-

bin, without a corresponding reduction in the number of cells, is sometimes called "oligochromæmia," or "chloro-anæmia."

The amount of hæmoglobin may be considered with reference to the blood as a whole and with reference to the individual corpuscles. As a whole it may be expressed in percentages, being normally about 12.5 to 13.75 per cent of the blood by weight; in hæmatological work the hæmoglobin when normal is commonly expressed as being 100 per cent, and variations from the normal are recorded in percentages with this as the standard. The color of the blood varies with changes in the amount and with chemical changes of the hæmoglobin, and affords a colorimetric method of estimating it. The specific gravity of the blood also corresponds closely with the amount of hæmoglobin.

The hæmoglobin can also be considered with reference to the amount in each red corpuscle. The total percentage of hæmoglobin and the number of corpuscles being normal, each corpuscle may be regarded as possessing a normal amount of hæmoglobin. If the total proportion of hæmoglobin is relatively less than the number of corpuscles, each corpuscle possesses less than its normal amount of hæmoglobin, and these variations referred to the individual cells have a distinct clinical significance. The corpuscular quantities of hæmoglobin are quantitatively expressed by means of ratios, determined by dividing the total percentage of hæmoglobin by the percentage to normal of the number of corpuscles present. This ratio is variously termed the "corpuscular hæmoglobin ratio," the "globular value," "color index," etc. When the ratio is 1, each cell possesses its normal quantity of hæmoglobin, even though the number of corpuscles is abnormal. When, as often occurs in anæmic conditions, the ratio is less than 1, each cell possesses less than its normal share of hæmoglobin. The ratio rarely exceeds 1, as in pernicious anæmia and new-born infants.

When the red corpuscles are individually deficient in hæmoglobin (their ratio being less than 1), their color is correspondingly paler, often to a degree distinctly and markedly perceptible in microscopic examination; in such cases the thin centre of the corpuscles may be entirely colorless, only the thicker periphery exhibiting a pale color.

The composition and properties of hæmoglobin, and the chemical combinations and transformations to which it is subject

(as oxyhæmoglobin, methæmoglobin, hæmatin, hæmin, hæmatoidin, hæmatoporphyrin, carbon monoxide hæmoglobin, etc.), are interesting and important subjects which cannot be considered here. The most important constituent of hæmoglobin is iron, which also occurs in the blood in other combinations. Under pathological circumstances hæmoglobin may pass from the corpuscles into solution in the plasma, producing hæmoglobinæmia.

The shape of the red corpuscles is normally circular and disc-shaped, biconcave. The deviations from the normal form may be considered under three classes: (a) Crenation and other non-pathological changes in form; (b) endoglobular changes (vacuolation); (c) pathological changes in form, or poikilocytosis.

Crenation occurs in blood removed for examination, and is caused by increase of density of the plasma, by evaporation from exposure to the air or by the solution of additional substances in it. The corpuscles contract from loss of part of their fluid contents through osmosis; they become spheroidal or irregular in shape, with rounded or spiny projections. When the density of the plasma is diminished, as by the addition of water, the red cells absorb fluid by osmosis, swell, become spherical, faint, and colorless, the hæmoglobin passing into the plasma; these are the "shadow corpuscles." Portions of the corpuscles often become broken off, assuming a rounded form, and appear like minute corpuscles. Sometimes the corpuscles appear bent, twisted, or doubled up irregularly. Familiarity with the changes of form which erythrocytes may manifest under the artificial conditions to which they are exposed during examination is necessary to prevent mistaking them for pathological alterations.

Often spaces or *vacuoles* appear in the interior of the red corpuscles, from shrinkage of their substance; the vacuoles may be single or multiple, large or small. Usually they are artefacts and not pathological, but possibly at times they may be of pathological nature.

In some diseased conditions, especially in anæmias, the red corpuscles, or many of them, may be markedly abnormal or deformed in shape. Such abnormally shaped corpuscles are called **poikilocytes**. Sometimes, as often in pernicious anæmia, the corpuscles generally are elliptical or elongated in form; in other cases the poikilocytes are of various irregular shapes.

The size of the red corpuscles is normally 7 to 8 (averaging 7.5) micromillimetres in diameter and about 2 in thickness. Under normal conditions the size of the corpuscles may vary within small limits, either in the same individual or in different individuals. Greater variations in size occur in pathological conditions, especially in anæmias. Abnormally large cells are termed **macrocytes**, abnormally small cells **microcytes**. In some cases the cells are generally and almost uniformly, or on an average, undersized or oversized; sometimes in the same case the cells exhibit considerable variations in size. Macrocytes are especially frequent in pernicious anæmia; while in chlorosis microcytes are quite characteristic.

Nuclei.—Human red blood corpuscles are normally devoid of nuclei, except at an early embryonic period. In certain diseases,

especially leukæmia and severe anæmias, nucleated red cells may be present either in small numbers or in small proportion to the non-nucleated cells. Three varieties of nucleated erythrocytes are distinguished, according to the size of the corpuscle: (*a*) **normoblasts**, nucleated corpuscles of the normal size of red corpuscles; (*b*) **megaloblasts**, nucleated cells of abnormally large size; (*c*) **microblasts**, nucleated cells of very small size.

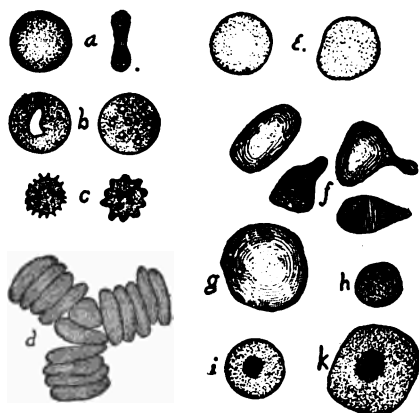


FIG. 2.—Normal and Abnormal Red Blood Corpuscles. *a*, Normal corpuscles, side and edge view; *b*, vacuole formation; *c*, crenated corpuscles; *d*, rouleau formation; *e*, pale corpuscles, deficient in hæmoglobin; *f*, polkilocytes; *g*, macrocyte; *h*, microcyte; *i*, normoblast; *k*, megaloblast.

Normoblasts are considered to be closely related to the normal erythroblasts of the bone marrow, from which the red blood corpuscles are derived; the nucleus is conspicuous, single, and rounded, sometimes divided into segments, or karyokinetic, stains deeply with nuclear and basic stains, and is frequently eccentrically situated. Normoblasts occur regularly in severe anæmic conditions (except chlorosis), and are abundant in sple-

nic leukæmia; they have no specially evil significance in such cases, and indeed indicate hæmopoietic activity.

Megaloblasts are very large erythrocytes, 11 to 20 micromillimetres in diameter, often somewhat irregular in outline and with a polychromatophilic tendency, and with a large, rounded, pale-staining nucleus. They approach the type of erythroblast occurring in early embryonic life. They may be found in most classes of cases in which normoblasts occur, but usually in much smaller number than the latter; they are especially abundant and characteristic in pernicious anæmia. When they are relatively abundant, they are regarded as of grave prognostic significance, representing an embryonic or inadequate process of blood regeneration.

Microblasts are very rarely encountered; they are abnormally small nucleated red corpuscles.

Staining Characteristics.—Red blood corpuscles are normally oxyphile in their staining affinities, taking acid stains like eosin or picric acid. In some abnormal conditions, especially severe anæmias, instead of being colored by the ordinary stains in the usual manner some of the corpuscles wholly or in part assume peculiar and unusual colors. This abnormality of the staining reaction is termed **polychromatophilia**, and the cells exhibiting the change are called *polychromatophiles*. Polychromatophilia indicates a chemical or degenerative alteration in the red corpuscles; it is one of the less common abnormalities of the erythrocytes, and usually occurs only in very severe anæmias.

A form of polychromatophilia ("granular degeneration") is rarely observed in which small, strongly basophilic granules are scattered about in red corpuscles, which may themselves be of normal or polychromatophilic coloration. These granules are probably a peculiar form of degeneration, and appear in lead poisoning and other conditions.

In the blood of diabetics the red corpuscles lose their affinity for the acid stains which they ordinarily take with avidity, and, on the contrary, stain with dyes which do not color normal red cells.

Rouleau Formation.—When a sufficiently thick layer of fresh blood is placed under the microscope, the red corpuscles normally collect temporarily in rouleaux, becoming arranged or packed together with their sides in contact, like a pile of coins. Patho-

logically the tendency to rouleau formation may be absent, as frequently in pernicious anæmia.

Many of the abnormalities of erythrocytes just considered, as variations in size, shape, hæmoglobin, and staining affinities, may be regarded as degenerative conditions of the corpuscles, arising from injurious or toxic alterations of the plasma or from diminished power and inability of the hæmopoietic agencies to produce healthy corpuscles. Other indications of degeneration may occur. The consistency or firmness of the corpuscles may be lessened, so that they change their shape at the slightest disturbance; the cells may become almost semi-fluid, so as to be hardly capable of retaining any definite shape, and almost run together in a gelatinous mass. The power of the erythrocytes to withstand changes in the density of the plasma (their "tonicity" or "isotonicity") may be lessened so that they more easily suffer osmotic changes, causing loss of hæmoglobin, change of shape, or other destructive alterations. The corpuscles may undergo irregular contractions or movements, or rupture, or fragments may break off.

Leucocytes.

Human leucocytes are of several varieties, differing in some characteristics, but similar in their general features. They are spherical when quiescent, irregular and variable in form during amœboid movement. They range in diameter from about 7 to 20 micromillimetres. They are of firmer structure and more vigorous vitality than the erythrocytes, not so easily influenced by changes in their environment, and not so subject to degenerative changes. They occur not only in the blood (blood leucocytes), but also in the lymphatic structures (lymphatic leucocytes), in the bone marrow (marrow leucocytes), in the spleen (spleen leucocytes), in the interstices of tissues (wandering cells), in pus (pus leucocytes), etc. They are typical cells, of active vitality. Their nuclei vary in the different varieties of leucocytes as to number, form, size, and staining properties. The cytoplasm or cell body is typical protoplasm, in which can be distinguished, especially during amœboid movement, a clear, hyaline, fluid, homogeneous peripheral portion, the hyaloplasm, and a more central granular portion, the spongioplasm. The cell-bodies of most white blood corpuscles contain protoplasmic gran-

ules, which vary in kind and present important distinguishing characteristics in the different varieties of leucocytes. These granules are distinguished chiefly by their size and staining affinities.* The two principal varieties in human blood are fine neutrophile granules and coarse oxyphile granules (ϵ and α granules of Ehrlich's classification); fine basophile (δ) granules occur but are inconspicuous and unimportant, while coarse basophile (γ) granules characterize mast cells. The granular appearance of fresh leucocytes, which often obscures the nuclei, is cleared away on addition of acetic acid, leaving the nuclei prominent as two or three spherical bodies; this is a useful means of recognizing and demonstrating leucocytes.

Leucocytes exhibit amoeboid movement in a very typical manner, and they possess the power of phagocytosis, as may be observed especially in connection with the malarial parasite.

The number of leucocytes in given volumes of blood in normal conditions ranges from 6,000 to 10,000 to the cubic millimetre; 8,000 is about the average standard. The number in the peripheral blood varies widely in different pathological conditions, ranging from 419, probably the lowest number on record (Cabot), up to 1,500,000 per cubic millimetre, and these variations are of great diagnostic significance. The deviation in number may consist in a parallel variation of all the varieties of leucocytes, or in an increase or decrease of single varieties. Increase in the number of the leucocytes is termed "leucocytosis" (also "hyperleucocytosis"), decrease in their number below the normal is called "leucopenia" (also "hypoleucocytosis").

The leucocytes are increased (leucocytosis) in numerous conditions. The number is higher in those in vigorous health and well-fed condition. At the height of digestion, as three or four hours after a rich proteid meal, there is ordinarily a temporary increase in their number of from 30 to 50 per cent; this is called the "digestion leucocytosis." Digestion leucocytosis is absent in some conditions, as in gastric carcinoma, other gastro-intestinal disorders, and occasionally in other conditions, so that its occurrence may be a matter of clinical significance. Exercise, massage, and brief cold baths cause a transient increase of leucocytes,

* Basophile granules are those which stain with basic stains, like methylene blue; oxyphile granules are those which take acid stains, as eosin, acid fuchsin; neutrophile granules are those which take neutral stains.

perhaps from vascular contraction and concentration of the blood. In young infants, especially the first week or two, the number is high (up to 30,000 or more). In the latter part of pregnancy, especially in primiparae, and after delivery, leucocytosis occurs. In the moribund period there is often a leucocytosis. The leucocytes may be increased after the administration of certain drugs and poisons, as ether, salicylates, pilocarpin, illuminating gas. After hemorrhage leucocytosis appears very quickly, within an hour or so. In most inflammatory and infectious diseases, and especially in suppurative conditions, marked leucocytosis appears and affords valuable diagnostic information. In pneumonia the leucocytosis may be extreme, the number occasionally reaching 100,000. In cases of infectious disease, when the infection is very mild or when it is overwhelming, without a vigorous bodily reaction, there may be no leucocytosis, but where the infection is moderate, so as to permit a vigorous body reaction, the leucocytosis is marked.

In uncomplicated tuberculosis, typhoid fever, malaria, measles, and influenza, leucocytosis is conspicuously absent,—a negative point that is sometimes of diagnostic significance. In malignant disease, carcinoma and sarcoma, leucocytosis is usually present, and may be extreme (up to 100,000). Leucocytosis may be present also in various chronic affections. The greatest increase in the number of leucocytes occurs in leukæmia, in which they may exceed 1,000,000 cells per cubic millimetre.

The leucocytes are diminished (leucopenia) in certain cases. In conditions of lowered health, debility, malnutrition, insufficient food, and starvation the leucocytes are decreased, sometimes below 1,000. Short hot baths and prolonged cold baths decrease the leucocytes, probably from dilatation of vessels and dilution of the blood. Pernicious anæmia usually exhibits marked leucopenia, the corpuscles sometimes falling below 1,000. Infectious diseases in which leucocytosis is absent, as tuberculosis and typhoid fever, may show a diminution in the number of leucocytes. In leukæmia complicated by infectious disease leucopenia may result.

The proportion of white to red corpuscles is ordinarily in the neighborhood of 1 to 600. When the two kind of cells increase or decrease proportionately, this ratio continues; but when they do not vary in correspondence, the ratio is altered accordingly.

In leukaemia the ratio of white to red cells may go as high as 1 to 4, or the white may even exceed the reds in number.

As to the **distribution of the leucocytes** in the blood, many authorities believe that they do not always occur in equal quantities in all parts of the circulation. In some forms of leucocytosis, for instance, it is believed that the leucocytes are aggregated in the peripheral circulation, the internal structures being correspondingly depleted, so that there is no alteration in the total number of leucocytes in the entire body. In many cases, however, there is doubtless an actual increase in the total number of leucocytes. Whether the peripheral blood, which alone is available for examination, is representative of the entire blood or not, the fact remains that marked and characteristic changes occur in the numbers of leucocytes there present, and that these changes are of valuable diagnostic significance.

Varieties of Leucocytes.—The leucocytes that occur normally or abnormally in human blood are of a number of different kinds, varying as to size, nuclei, granules, and staining properties. These varieties are small mononuclear leucocytes, large mononuclear leucocytes, transitional leucocytes, polynuclear leucocytes, eosinophile leucocytes, neutrophile myelocytes, eosinophile myelocytes, and mast cells, besides occasional atypical and as yet unclassified leucocytes.

Small Mononuclear Leucocytes (or small lymphocytes).—These are small spherical corpuscles about 6 to 8 micromillimetres in diameter. They are made up chiefly of a nucleus, which is surrounded by a narrow shell of body protoplasm. The nucleus is spherical and usually stains deeply with nuclear and basic stains. The body protoplasm stains but faintly, and is usually non-granular, though sometimes basophile granules can be demonstrated.

Large Mononuclear Leucocytes (or large lymphocytes).—These are large corpuscles, 12 to 15 micromillimetres in diameter. They contain each a single, large, rounded, pale-staining nucleus, which is surrounded by a large cell-body of faintly staining protoplasm that is non-granular or contains only inconspicuous basophile granules.

Transitional leucocytes are like the large mononuclear leucocytes in all respects, except that the nucleus instead of having a circular outline is indented at one side, giving it a horseshoe or

U shape. The cytoplasm also sometimes contains a few neutrophile granules.

The small and large mononuclear and transitional leucocytes are closely related to one another, forming together a well-defined group, the "lymphocytes," and are regarded by some as younger stages of leucocytes. Intermediate forms, as to size and nuclei, may at times be observed between all three varieties, so

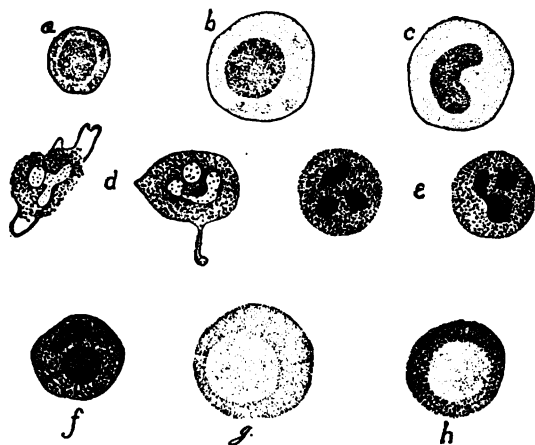


FIG. 3.—Human Leucocytes. *a*, Small mononuclear leucocyte, stained appearance; *b*, large mononuclear leucocyte, stained; *c*, transitional leucocyte, stained; *d*, polynuclear leucocytes, living amoeboid appearance; *e*, polynuclear leucocytes, stained; *f*, eosinophile leucocyte, stained; *g*, myelocyte (neutrophile), stained; *h*, eosinophile myelocyte, stained.

that sometimes sharp dividing lines between the three can hardly be drawn. Transitional leucocytes especially are often to be regarded as simply a variety of the large mononuclear group.

Polynuclear (polymorphonuclear or neutrophile) **Leucocytes**.—This corpuscle is about 10 micromillimetres in diameter. It has a conspicuous body of vitally active protoplasm containing large numbers of fine (ϵ) neutrophile (or rather, perhaps, faintly oxyphile) granules. The nucleus stains deeply, and is very variable and irregular in shape, because of which the term "polymorphonuclear" is often applied to this form of leucocyte. On treating the fresh leucocyte with acetic acid, the cell-body clears up and the nucleus stands out as about three separate spherical bodies, whence the name polynuclear applied to this variety. Instead of possessing three separate nuclei, however, it is generally believed that this leucocyte has only a single nucleus, very irregu-

lar, twisted or lobed in form, often having the appearance of being divided into separate parts, which are, however, united by strands of the nuclear substance. This is the most abundant leucocyte of normal human blood, and exhibits very active amoeboid movements and phagocytosis. The corpuscles of pus are identical with polynuclear leucocytes.

Eosinophile Leucocytes.—These are about 10 micromillimetres in diameter. They have a rather large irregular and polymorphous nucleus, or perhaps two or three separate rounded nuclei, staining less deeply with basic stains than the nucleus of the preceding variety. The cell-body is crowded with conspicuous coarse oxyphile (α) granules, which stain intensely with acid stains such as eosin (whence the name eosinophile); the characteristic granules enable this variety of leucocyte to be very distinctly recognizable, both in the fresh and stained condition.

Neutrophile myelocytes are found in the blood only in abnormal conditions; they (or corpuscles very similar) occur normally in the bone marrow. They are usually larger than other varieties of leucocytes, ranging from 20 down to 12 or 13 micromillimetres in diameter. They possess each a single large rounded pale-staining nucleus eccentrically placed, and the ample cell body is crowded with fine neutrophile granules. Myelocytes are perhaps intermediate forms between large mononuclear and polynuclear leucocytes. They occur in the blood in small number in pernicious anæmia, chlorosis, and other anæmic conditions, and in some leucocytoses; but they are especially abundant and characteristic in splenic leukæmia, in which they form a large percentage of the leucocytes present.

Eosinophile Myelocytes.—These may occur in the blood in any condition in which the neutrophile myelocytes appear (chiefly in splenic leukæmia and occasionally in pernicious anæmia) but in much smaller number than the latter. They resemble the ordinary neutrophile myelocytes in every respect except that their cell bodies are crowded with coarse oxyphilic granules instead of neutrophile granules.

Mast Cells.—These cells ordinarily occur in the body tissues, but occasionally they find their way into the normal circulating blood. In splenic leukæmia they may be present in the blood in large numbers. They are usually large cells, ranging up to 20 or 30 micromillimetres in diameter, each with an irregular or lobu-

lar pale-staining nucleus, and coarse basophile (γ) granules in the body protoplasm.

Atypical Leucocytes.—Nearly all leucocytes observable in the blood are distinctly referable to one or another of the classes described. Occasionally, however, leucocytes may be seen with atypical characters, as to size, nuclei, granulation, etc., which do not permit them to be placed with any of the generally recognized classes,—such as mononuclear leucocytes with neutrophile granules, leucocytes with basophile granules, etc. Degenerating forms, as leucocytes with vacuoles, are also sometimes seen.

Basophile Granules.—It will be observed that the most abundant and important granules of human leucocytes are the fine neutrophile or oxyphile (ϵ) and the coarse oxyphile (α) granules. Basophile granules can often be demonstrated by proper methods, but they are ordinarily not very abundant or conspicuous, and little or no clinical importance is attached to them. They may be demonstrated sparingly in lymphocytes and myelocytes, are marked in mast cells, and may occur in atypical leucocytes.

One class of basophile granules that has been specially differentiated are the *perinuclear basophile granules*. These are basophilic granules that can sometimes be demonstrated immediately surrounding the nuclei of mononuclear and polynuclear leucocytes. Their clinical significance, if they have any, has not yet been settled.

Relative Proportions of Different Varieties of Leucocytes.—In normal blood the different varieties of leucocytes are present in tolerably constant proportions to one another, which are, in the adult, in the neighborhood of the following figures:

	Per cent.	No. per cubic millimetre.
Small mononuclear leucocytes.....	24	1,900
Large mononuclear and transitional leucocytes....	6	450
Polynuclear leucocytes.....	68	5,500
Eosinophile leucocytes.....	2	150
	100	8,000

Mast cells may also be present normally up to the proportion of about 0.5 per cent.

In abnormal conditions these proportions may undergo great

fluctuations. In leucocytosis and leucopenia all the varieties of leucocytes may be increased or diminished together, without any change in their relative proportions; or the change in the total number may be due to an increase or decrease in some particular variety of leucocyte, which would correspondingly modify the relative proportions of the different kinds. Also, the total number of leucocytes may be normal, and yet their relative proportions may be modified. All these variations are in many cases of highly important diagnostic significance.

The *mononuclear leucocytes*, large or small, or both, are increased ("lymphocytosis") relatively to the other varieties, either without or with an increase in the total number of leucocytes, in various conditions. In infants the lymphocytes normally are much more numerous (up to 50 or 60 per cent) than in the adult, at the expense of the polynuclears; this lymphocytosis subsides parallel with the development of the child. Often debilitated conditions in the adult present relative lymphocytosis. Lymphocytosis may also occur in chlorosis, pernicious anæmia, syphilis, thyroid disease, and in various other conditions. In lymphatic leukaemia the greatest increase in the mononuclear leucocytes occurs, both in relative proportion (over 90 per cent of all the leucocytes) and absolute numbers.

The *polynuclear leucocytes* may be relatively decreased where there is an increase in the proportions of other varieties. In infancy the polynuclears are relatively less than in the adult, ranging as low as 20 per cent. In most of the ordinary leucocytoses, especially those accompanying inflammatory and infectious processes, hemorrhages, and malignant disease, there is a relative and absolute increase of the polynuclears, these corpuscles being the ones chiefly concerned in the ordinary vital processes and reaction to disease. Even when the total number of leucocytes is not above normal, the relative proportion of polynuclears may be increased; such an increase has a similar significance to that of an absolute polynuclear leucocytosis.

The *eosinophile leucocytes* are diminished in relative number in many of the conditions presenting polynuclear leucocytosis. They are relatively and absolutely increased (an increase of these cells being called "eosinophilia") in infancy, trichinosis, asthma, certain skin diseases, some bone diseases (osteosarcoma, osteomalacia, etc.), in some cases of leukaemia, syphilis, nervous and

mental disease, chlorosis and pernicious anæmia, and in various other conditions without known cause. Their relative number may vary from a slight increase up to 6, 10, or 20 per cent, or even more; in one case of trichinosis 68.2 per cent of eosinophiles have been observed, and in another case 77.3 per cent.

Myelocytes are absent from normal blood. They occur occasionally in small numbers in many cases of severe anæmia and ordinary leucocytosis without any special evil significance. They are present in large proportion (averaging about 35 per cent) in splenic leukæmia; and their presence in large numbers is pathognomonic of this disease.

Mast cells and *basophile leucocytes* are occasional stray visitors to normal blood. In splenic leukæmia they may comprise up to 10 per cent of the leucocytes.

The percentages of the different kinds of leucocytes express only their relative number, and do not in themselves alone give any indication as to whether there is an absolute change in the number of the individual varieties of leucocytes per cubic millimetre. To determine the absolute variations of leucocytes it is necessary to calculate the number of the different kinds in each cubic millimetre, and compare this with the normal standard (such as that given on page 30). Thus, in a case of leukæmia exhibiting 100,000 leucocytes to the cubic millimetre, of which 50 per cent (50,000) were myelocytes, 35 per cent (35,000) polynuclears, 10 per cent (10,000) mononuclears, 2 per cent (2,000) eosinophiles, and 3 per cent (3,000) basophiles, all the varieties would be absolutely increased over their normal number per cubic millimetre, while in relative proportions the polynuclears and mononuclears would be diminished and the eosinophiles unchanged.

Blood Plates.

The blood plates are small, colorless, homogeneous, clear, hyaline, spherical bodies, about 3 or 4 micromillimetres in diameter. They very readily disintegrate and disappear when the blood drop is exposed to the air, so that they are not usually to be seen in specimens of blood unless special precautions are taken to preserve them. They occur singly, or aggregated in clusters, often surrounded by or in the neighborhood of granular matter, probably the debris of disintegrated blood plates. They number

about 300,000 or 400,000 to the cubic millimetre of blood. Their number, nature, origin, and purpose are not positively known. They fluctuate in various disease conditions, but as their examination is somewhat difficult and unreliable, and as their pathological significance has not been definitely determined, they are not generally regarded or utilized in clinical work.

Parasites.

The parasites that may occur in the blood are both vegetable and animal. The **vegetable blood parasites** are certain of the common pathogenic and especially septicæmic bacteria (such as typhoid, anthrax, and influenza bacilli, streptococci, staphylococci, pneumococci), and the spirillum of relapsing fever. The blood is an unfavorable and antagonistic medium for ordinary bacteria, and while they must be present in the blood stream to some extent, their numbers are relatively so small that the chances of finding them in specimens of blood taken for examination are ordinarily very slight. Ocular examination of the blood for these bacteria is therefore usually negative; they can be adequately searched for only by cultural methods, employing a considerable quantity of blood for the purpose.

The **spirillum of relapsing fever** (*spirochæta Obermeieri*) may be easily found in the circulating blood, sometimes in large numbers, during and for a day or two before the febrile paroxysms of this disease. They are long, slender, wavy, or spiral filaments, 30 or 40 micromillimetres long, actively motile. They are best observed in fresh blood, but may also be stained.

The **animal parasites** of human blood are the parasite of malaria, the *filariæ sanguinis hominis*, and the *schistosoma hæmatobium*.

Parasite of Malaria.—The malarial parasite is a protozoan living in human blood. The full life history of the parasite has not been ascertained; its period of existence in the human blood is only a portion of its life cycle. A prominent if not the only agency by which the parasite is introduced into the human body appears to be by means of certain species of mosquitoes (of the genus *Anopheles*); some observations have been made as to the life of the parasite in the mosquito, but nothing else is known as to the extra-human existence of the organism.

The parasite under consideration is associated specifically and exclusively with malarial disease, and its detection in the blood is absolutely pathognomonic of malaria. Three distinct varieties of the malarial parasite and three corresponding types of malarial disease are distinguished, namely, the tertian, quartan, and æstivo-autumnal forms. These three are practically distinct varieties, not merging into one another (except possibly in exceptional instances).

1. The **tertian parasite** in the human blood goes through a life cycle of about forty-eight hours. Its earliest forms appear during the latter part of or soon after the chill or paroxysm as small, colorless hyaline bodies within and occupying a small part of the red blood corpuscles, but not showing the complete lack of color and sharp outlines characteristic of vacuoles in these corpuscles. These intracorpuseular bodies usually exhibit active amœboid movements, a feature distinguishing them from vacuoles. As time passes they increase in size, and in a few hours numerous small pigment granules appear within the parasites, dark in color, variable in size and shape, sometimes appearing as minute rods, sometimes as irregular fragments. The granules are distributed irregularly through the parasite, and are generally in very active dancing motion. With the growth of the organism, the infected red corpuscle becomes paler and increased in size. As the parasite approaches maturity its amœboid movements decrease, and the pigment, which may be still in active motion or may become quiescent, tends toward a peripheral arrangement. Toward the end of forty-eight hours the organism attains full growth, has become about the size of a normal red corpuscle, and about it may be traced only the pale, narrow margin of the expanded red disc.

After full size is reached the parasite in its further evolution may pass through a number of phases; it may (*a*) undergo segmentation, (*b*) become a free extracorpuseular form, exhibit (*c*) fragmentation or (*d*) vacuolization, or (*e*) become flagellated.

Segmentation is a process by which each parasite divides into a number of spores from which a new generation of the protozoan is developed, and is perhaps the normal course of the matured parasites. It occupies a comparatively brief time; the chill or paroxysm occurs simultaneously with the process of segmentation. The process begins by the pigment becoming col-

lected in the centre of the parasite in coarse, motionless granules. The protoplasm of the organism, which was previously clear, glistening, and hyaline, loses its lustre, becomes less refractile, and acquires a finely granular appearance; in it radiating lines gradually develop, dividing the organism into segments. The fully developed segmenting body consists of a central collection of pigment surrounded by twelve to twenty separate radiating protoplasmic segments in a rosette or daisy-like form, each ray

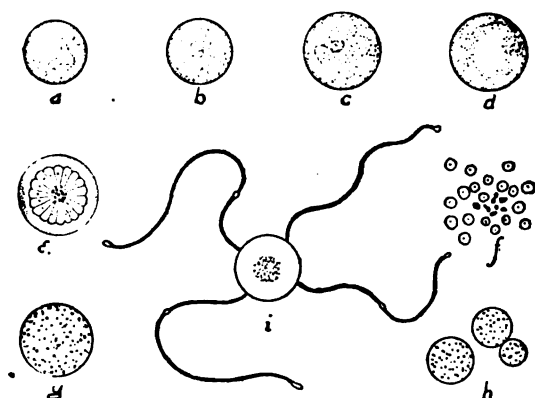


FIG. 4.—Tertian Malarial Parasite. a, Young hyaline intracorpuseular form; b, young pigmented actively amoeboid form; c, half-grown form; d, maturing form; e, segmenting form; f, segments and pigment free in the plasma, after segmentation; g, free extracorpuseular form; h, fragmenting form; i, flagellated form.

exhibiting a faint punctate marking in its centre. The remains of the red corpuscle may or may not be visible about the parasite. The segmenting bodies sometimes, however, do not present quite such regular figures. A little later the segments become entirely separated from one another and appear as small spherical hyaline bodies free in the plasma, with the collection of pigment also free in their midst or in their vicinity. The hyaline bodies are then supposed to make their way into red corpuscles and begin a new cycle of intracorpuseular existence.

Sometimes, instead of segmenting, the parasites, having escaped from or completely destroyed the surrounding red corpuscles, appear in the plasma as free or *extracorpuseular forms*. These are spherical or rounded in shape, clear, hyaline, and glistening in appearance, and studded with pigment granules, which are usually quiet, though sometimes in motion; they are of about

the size of red corpuscles, and do not exhibit amœboid movements. Sometimes half-grown intracorpuseular organisms leave their hosts and become free. The extracorpuseular parasites sometimes slowly disintegrate and disappear ("cadaveric forms"); sometimes they exhibit fragmentation, vacuolation, or flagellation.

Fragmentation is a division by the process of budding not infrequently manifested by the free extracorpuseular forms. A rounded prominence grows out from the margin of one of these forms, increases in size (at the expense of the parent cell), and is finally severed from its parent as a smaller spherical separate pigmented body. Four or five smaller forms may be thus derived from one large one, sometimes connected for a time by filaments.

Rarely the large free parasites become *vacuolated*, a number of round vacuoles appearing within them.

The *flagellated forms* of the parasite are very striking and interesting objects. In different countries they are observed with different degrees of frequency, but in this vicinity they are seen in only about four or five per cent of the cases. They do not appear immediately after withdrawing the blood from the body, but develop after the lapse of some time, ten minutes or more. The large, free, extracorpuseular bodies are the forms which become flagellated. Their pigment becomes exceedingly active and collects in the centre of the parasite, while from the periphery are protruded from one to five thread-like processes or flagella, in length several times the diameter of the organism, and usually possessing slightly bulbous extremities or slight bulbous expansions in their course. These flagella whip about with vigorous undulating motions and often cause a violent commotion among the neighboring red corpuscles. Undulating movements of the periphery of the organism are also observable, and frequently the flagella break off and, still undulating, make their way about in the blood. The flagellate movements cease and the flagella disappear after a few minutes. The significance of flagellation in these parasites is not well understood; the process may be a stage in the extracorporeal existence of the parasite, or the flagella are perhaps a form of spermatozoon and associated with reproductive processes.

Infection by the tertian parasite may consist in the presence

of one set of organisms ("single tertian") or of two sets ("double tertian"). All the parasites in each set begin their development at the same time, pass simultaneously through all the stages of their 48-hour life cycle, and arrive at maturity and undergo segmentation together. The parasites do not exhibit all stages of growth at one time, but all the individuals of each group are always in like stages of development; only in double infections two stages of growth are visible at the same time, the individuals of one set being full grown, while those of the other set are half grown. In single tertian infections a paroxysm occurs every other day; in double tertian one set of parasites segments each day, and consequently a chill occurs daily. The tertian parasite produces typical intermittents and milder forms of malarial fever, and is very susceptible to the action of quinine. It is a common form of malarial infection, and the only form indigenous in the northern parts of the United States.

2. The **quartan parasite** is rare in this country, occurring in about one per cent of the cases of malaria in the vicinity of Baltimore; it is commoner in some other countries. Its life cycle is about seventy-two hours. Its appearance and course of development are similar to those of the tertian parasite, but there are certain well-marked points of difference. The protoplasm of the growing forms of the quartan parasite has a sharper, more refractive appearance, the amœboid movements are less marked, the pigment is coarser and less actively motile, the infected red corpuscles are not enlarged or decolorized. The mature parasites are somewhat smaller than the red corpuscles. Segmentation is often preceded by a radiating or star-shaped arrangement of the pigment, which seems to pass from the periphery to the centre by definite channels. The segmenting forms divide into six to twelve segments or rays, frequently exhibiting exquisite symmetrical forms. Instead of segmenting, the quartan organisms may become free in the plasma or undergo fragmentation, vacuolization, or flagellation in a manner similar to the tertian organism. One, two, or three sets of quartan parasites may be present, each ripening on a separate day and forming a single, double, or triple quartan infection, with a chill every three days, two chills in three days, or a chill daily.

3. The **æstivo-autumnal parasite** is associated with the more severe, the more irregular and protracted, the continued and remit-

tent, the chronic and cachectic, and the more malignant forms of malarial disease, and is the most resistant to quinine. This form of malaria is abundant toward the tropical regions, gradually diminishes northward, and does not extend so far north as the tertian form. The earliest visible forms of the æstivo-autumnal parasite are small non-pigmented hyaline bodies in the red corpuscles, rounded, irregular, or often characteristically ring-shaped, and usually showing active amœboid movement. These forms are usually smaller and more hyaline than the corresponding tertian forms. These hyaline bodies gradually enlarge and fine pigment granules develop in them, not so numerous as in the tertian variety, and showing but little motion. In this stage the diameter of the organism usually does not exceed one-third that of the red corpuscles. The infected corpuscles are frequently

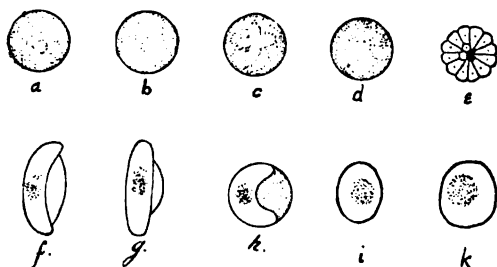


FIG. 5.—Æstivo-autumnal Malarial Parasite. *a, b, c, d*, Young intracorporeal forms; *e*, segmenting form; *f*, crescent form; *g*, elliptical form; *h*, crescent form within red corpuscle; *i, k*, ovoid forms.

brassy or deepened in color, shrunken, or their substance is retracted about the parasite. As the organism approaches maturity the pigment collects in the centre in solid clumps, and the protoplasm becomes peculiarly refractive; these are the presegmenting forms and occur only just before or during the paroxysm. The segmenting forms are closely similar to those of the tertian variety, except that they are much smaller. Segmentation of the æstivo-autumnal parasite takes place in the spleen or other internal organs, and during this process the parasites are retained within the viscera. Before and during æstivo-autumnal paroxysms, therefore, no parasites at all may be visible in the peripheral blood, and segmenting forms especially are seen only in very severe cases.

Extracorporeal free forms of this parasite are common,

round, ovoid, or crescentic in shape. The crescentic and related ovoid organisms are very striking and distinctive forms of the æstivo-autumnal parasite, not occurring in other varieties of malaria. They are crescentic or elliptical-shaped masses of clear hyaline protoplasm, containing near their centre a conspicuous collection of coarse, dark, motionless pigment granules or rods; a faint curved line can often be distinguished on the concave side of the crescents joining the two extremities, or on one side of the long elliptical forms. The length of the crescents is slightly greater than the diameter of the red corpuscles. The crescents develop from intracorpuseular forms, and may occasionally be distinctly seen within red corpuscles; often, especially in stained specimens, the remains of a red corpuscle may be seen attached to the crescents; and the faint curved line is a last vestige of the red corpuscle. Further than that they are a phase of the æstivo-autumnal parasite, little is really known as to the nature and significance of the crescents. They appear in æstivo-autumnal cases only after the lapse of several days from the onset of the disease; they are very persistent, resistant to treatment, and may be present alone in the blood for a long time without giving rise to paroxysms or definite symptoms, though relapses may occur attended with the advent of the hyaline forms.

The rounded extracorpuseular free æstivo-autumnal parasites may undergo fragmentation and flagellation in a manner precisely similar to the tertian forms, and both round and crescentic forms may become vacuolated. The life cycle of this variety of malarial parasite is not definite, but ranges from twenty-four to forty-eight hours. Several sets of the parasite may be present. The overlapping of the periods of segmentation of the several sets may cause continuous febrile manifestations without regular periodicity; and the clinical manifestations of this infection are very various.

Mixed infections occasionally occur, in which two varieties of the parasite, as the tertian and æstivo-autumnal, are present in the blood at the same time.

Phagocytosis is frequently observable in connection with the malarial parasite, in a very striking and typical way. The leucocytes can be seen to approach free parasites and engulf them within their substance, where the parasite after a time disintegrates and disappears.

The pigment left after breaking up of the segmenting forms remains free in the plasma for a time as conspicuous coarse black particles, constituting the condition known as, "melanæmia." Leucocytes may also be seen containing similar pigment particles which they have taken up as such or which remain from disintegrated engulfed parasites; these are called "melaniferous leucocytes." The presence of pigment in the plasma or leucocytes has an intimate association with malaria, that was recognized years before the parasite was discovered.

A point that has not been much studied in connection with the parasites is that relating to the septenary periodicity of malaria, the tendency of the paroxysms to recur after intervals of twenty-one, fourteen, or other multiple of seven days.

The examination of blood for the malarial parasite is a simple and satisfactory procedure. The blood may be examined either fresh or stained, but the former is much the easier and more satisfactory, as the parasites are as conspicuous when fresh as when stained, and in the preparation of stained specimens many parasites disappear. The tertian parasites are most conspicuous during the chill, the æstivo-autumnal forms during the intermission, but the examination may be made at any time (except that æstivo-autumnal forms are apt to disappear during the paroxysm). The examination should be made before quinine is administered, as that drug often very quickly clears the parasites from the blood. The objects to be searched for are the parasites, also pigment in the plasma or leucocytes. All the pigmented forms of the parasite are conspicuous and can be distinguished with ease and absolute certainty; the variety present, the stage of growth, and usually the number of infecting sets are recognizable, and usually the day when the next chill is to be expected can be foretold. The small, non-pigmented, intracorpuseular hyaline forms are much less easy to recognize with certainty or to distinguish from vacuoles; the most reliable differential point is the occurrence of amœboid movement in the parasite, though even vacuoles may undergo changes of form.

Filaria Sanguinis Hominis.—The filariæ found in the circulating blood of man are embryonic forms of a parental nematode worm which is lodged at some point in the lymphatics or tissues. Four varieties are known—*filaria diurna*, *filaria perstans*, *filaria nocturna*, and *filaria Demarquaii*, similar in their general feat-

ures, but with specific differences. The parental form of the *filaria nocturna* is the *filaria Bancrofti*; that of *filaria diurna* is supposed to be the *filaria loa*; those of *filaria perstans* and *filaria Dêmarquaii* are unknown. The *filaria nocturna* is the only variety which has been found in the United States.

The *filaria diurna* is present in the peripheral blood chiefly during the daytime, the *filaria perstans* continuously; both occur in Western Africa. These *filariæ* may affect the body for years, often without giving rise to appreciable symptoms, but in some cases producing marked disturbances. The *filaria diurna* is not known to produce pathological consequences; *filaria perstans* in many individuals causes no morbid symptoms, but in others is perhaps etiologically associated with the Congo sleeping sickness. *Filaria Dêmarquaii* has been differentiated in blood (both nocturnal and diurnal) from a few individuals of Saint Vincent, an island in the West Indies, and possibly of the Niger region also; little is known about this parasite. In one case rhabditiis Niellyi, an immature nematode related to *filaria*, has been seen in the blood.

The *filaria nocturna* is common in the tropics, and numerous cases indigenous in the southern half of the United States have been reported. The parent form of this worm, called the *filaria Bancrofti*, is about 94 millimetres long by .185 millimetre in diameter; it is lodged in the lymphatic vessels about the genito-urinary organs and lower extremities, and causes obstruction of the lymph circulation, manifested by chyluria, lymph scrotum, filarial abscesses, elephantiasis, and other conditions. The parent female discharges large numbers of small embryonic forms, the *filaria nocturna*, which enter the circulating blood, and are also frequently found in the urine and pus from abscesses.

The *filaria nocturna* is found in the blood only or chiefly in the nighttime, although it may be found in the daytime after prolonged rest in bed. They are about .3 millimetre long and



FIG. 6.—*Filaria Sanguinis Hominis Nocturna*, in the blood, magnified 400 diameters. From photograph of living specimen. (F. P. Henry.)

.0075 millimetre in diameter, and therefore small enough to pass through the capillaries. They appear in the blood in comparatively small numbers, so that it may be necessary to examine a large quantity of blood in order to find them. They have a slender, worm-like shape; the posterior extremity is tapering and pointed, the anterior end rounded and blunt. The embryo is enveloped in a delicate sheath. The live worm exhibits active wriggling motions, but without much progressive locomotive movement. In microscopical specimens it retains its vitality and motility for a long period, and is remarkably resistant to cold. The mosquito is supposed to be concerned in the life history of filariæ, taking them from man and ultimately depositing them in water, from which they are ingested by the human subject.

Schistosoma hæmatobium.—This is a trematode worm occurring chiefly in the veins of the pelvic viscera and giving rise to the disease called "bilharziosis." As the worms and their ova do not occur in the peripheral blood, their detection is not a matter of hæmatological examination.

In **variola and vaccinia** small actively amœboid bodies appear in the blood in small numbers, about 3 or 4 micromillimetres in diameter, some with granular protoplasm, some pale and containing a few dark pigment-like granules in their centre. In vaccinated children they appear about the sixth to the fourteenth day. They have been also observed in vaccinated calves and monkeys, and the granular body has been seen in the blood of normal children and monkeys. Whether these bodies are hæmocytes or protozoa has not been settled; but if they occur in normal blood they are doubtless hæmocytes.

B. TECHNIQUE OF BLOOD EXAMINATION.

The object of clinical hæmatological work is to determine the various features of pathological significance that have just been reviewed. As an aid to systematic examination and for recording and reporting the results, a printed blank form presenting the points searched for is useful, such as that on the following page.

To determine these features the following are the principal procedures in general use, besides which there are a number of unusual, difficult, or unsatisfactory methods rarely employed:

Obtaining the specimen of blood.

Macroscopic examination.

Estimation of hæmoglobin.

Enumeration of red corpuscles.

Date, ..., 190 .

EXAMINATION OF BLOOD.

Name of patient.....

Macroscopic appearance.....

Red blood corpuscles:

Number of red corpuscles per cubic millimetre.....(..per cent of normal).

Hæmoglobin.....per cent. Corpuscular hæmoglobin ratio.....

Macrocytes..... Microblasts.....

Microcytes..... Normoblasts.....

Polychromatophilia..... Megaloblasts.....

Poikilocytes.....

Rouleau formation..... Volume of red corpuscles.....

.....

.....

.....

Leucocytes:

Number of leucocytes per cubic millimetre.....

Proportion of white to red corpuscles, 1 to.....

Relative proportions of leucocytes:

Small mononuclear..... Polynuclear.....

Large mononuclear..... Eosinophile.....

Transitional..... Myelocytes.....

.....

.....

.....

Blood plates.....

Granules..... Pigment.....

Fat..... Specific gravity.....

Coagulation.....

Widal reaction: result.....; dilution, 1 to.....; time.....

Parasites.....

Malarial parasite.....

.....

.....

.....

Remarks.....

.....

.....

.....

(Signature)

Determination of corpuscular hæmoglobin-ratio.

Enumeration of leucocytes.

Determination of proportion of white and red corpuscles.

Microscopical examination of fresh specimens.

Microscopical examination of stained specimens, differential counting of leucocytes, etc.

Widal test for typhoid fever.

Determination of volume of red corpuscles.

Determination of specific gravity.

Bacteriological examination.

Chemical examination.

Micrometry.

Determination of coagulation time.

Study of blood plates.

Tests for blood.

The various points to be determined, as outlined in the blank form presented, are ascertained by the technical procedures mentioned, as follows:

Macroscopic appearance, by macroscopic inspection.

Number of red corpuscles per cubic millimetre, by enumerating them.

Amount of hæmoglobin, by special tests therefor.

Corpuscular hæmoglobin-ratio, by calculation from number of red corpuscles and amount of hæmoglobin.

Macrocytes, microcytes, polychromatophilia, poikilocytes, microblasts, normoblasts, megaloblasts, and rouleau formation, by microscopic examination of fresh and stained specimens.

Volume of red corpuscles, by centrifugal method.

Number of leucocytes per cubic millimetre, by enumerating them.

Proportion of white to red corpuscles, by calculation from the numbers of these cells.

Relative proportions of leucocytes, and number of nucleated red corpuscles, by differential count of stained specimens.

Blood plates, by examination of fresh or stained specimens, or by special methods.

Granules, pigment, and fat, by examination of fresh or stained specimens.

Specific gravity, by special methods.

Coagulation, by macroscopic examination, microscopic observation of fibrin formation in fresh specimens, or special methods.

Widal reaction, by special test.

Parasites, by examination of fresh or stained specimens, or (in the case of bacteria) by cultural methods.

In making a hæmatological examination all the apparatus and materials required for the tests proposed to be made should

be in readiness and convenient, so that the work necessary in the presence of the patient can be completed with despatch and with but a single puncture of the skin, which with nervous patients is quite an ordeal. The needle for puncturing the skin, a mixture of equal parts of alcohol and ether for cleaning the skin, sterile or clean gauze, water, the hæmoglobinometer and its capillary tubes, the pipettes for counting the red and white corpuscles, the diluting fluids, cleaned cover-glasses and slides, thumb forceps, or whatever is needed at the bedside in the tests to be made, should be at hand, ready for instant use. After duly procuring the specimens of blood, the outfit is taken to the laboratory and the remainder of the work done there.

Obtaining Specimens of Blood.—For ordinary purposes only a drop or two of blood is necessary or available. This is usually taken from the lobe of the ear, which is conveniently accessible and comparatively insensible to pain. To make the puncture a tiny lancet or a surgical needle with a cutting edge is employed; this should of course be aseptic when used. A straight Hagedorn needle about 5 centimetres long is very satisfactory for the purpose; this may be run through the cork of a vial of about 4 cubic centimetres capacity, which, filled with alcohol to keep the needle sterile, makes a very convenient receptacle or case for the needle, while the cork serves as a handle for the instrument (Fig. 7). Before making the puncture the skin should be cleaned and sterilized by rubbing it with gauze saturated with alcohol, or equal parts of alcohol and ether, and then dried. The needle is then plunged with a quick, firm motion into the skin, and on withdrawing it the slight puncture can be enlarged if necessary by means of the sharp cutting edge of the needle. The puncture should be large enough so that a drop or two of blood will emerge from it spontaneously, as if too much pressure is required to press the blood drop out lymph will also be forced out and affect the result. The needle prick causes very little pain, though some individuals will be nervous about it.



FIG. 7.—Hagedorn Needle in Vial of Alcohol, for use in obtaining specimens of blood.

Macroscopic Examination.—The appearance of the blood drop,

as it oozes out on the skin, can be noted. Usually it will appear normal; but marked variations of color may be perceptible to the naked eye, as undue pallor in severe anæmias, etc., or marked changes in consistency may be noted, as a thin and watery or a thick and tarry condition. The time required for coagulation to occur may be roughly estimated and any tendency to hæmophilia be detected.

Estimation of Hæmoglobin.—Most of the methods proposed for the determination of the hæmoglobin are colorimetric in char-

acter, carried out by matching the tint of a definite dilution of the blood with a standard color scale. According to another method, the hæmoglobin is estimated indirectly from the specific gravity.

Of several instruments introduced for the purpose the hæmoglobinometer of Fleischl is the one in most general use. Fleischl's instrument (Fig. 8) possesses a



FIG. 8.—Fleischl's Hæmoglobinometer. (Leitz.)

ated along one side (PP), to which is attached a wedge-shaped red glass slide (KK), thin at one end and increasing in thickness to the other end, so that all shades of red are presented along the slide from a deep red at the thick end to a very pale red at the thin end. By means of a sort of rack-and-pinion arrangement (R), worked by a milled disc (T), the sliding frame can be moved one way or the other and the scale can be read off at the mark M. On the stage of the instrument is a cylindrical metal receptacle G, with a glass bottom, divided into two compartments (a, a') by a vertical partition. Beneath the stage is a white reflector (S) by which light can be thrown upward through the receptacle G. The latter is so situated that the colored glass scale KK lies directly underneath the compartment a', while noth-

ing is interposed between compartment *a* and the reflector *S*. Accompanying the instrument is a small capillary glass tube of definite capacity, fastened in a metal handle (Fig. 9).

To make the estimation of hæmoglobin one end of this capillary tube is touched to the blood drop, when the blood by capillary action should promptly and immediately flow into and completely fill the tube. The capillary tube when used should be dry, as a trace of moisture will dilute the blood and impair the test; it should also be perfectly clean and free from oil (as by the use of equal parts of alcohol and ether), or the capillary action will be interfered with and the blood will not flow into it. The tube being completely filled, any superfluous blood on the outside should be removed, and the blood within it is entirely washed, by means of a jet of water from a pipette or medicine dropper, into the compartment *a* of the hæmoglobinometer. This compartment is then filled with water to the brim, the blood at the same time being thoroughly mixed with it by stirring with the handle of the capillary tube; the upper level of the fluid should form a convex surface rising slightly above the upper edge of the metal receptacle. The other compartment (*a'*) is then filled with water to the same height as compartment *a*, care being taken that the fluids on the two sides of the partition do not mix.



FIG. 9.—Capillary Pipette accompanying Fieschl's Hæmoglobinometer.

The estimation must be made by artificial light, such as a candle placed a short distance in front of the hæmoglobinometer, in a darkened room from which all daylight is excluded. This is necessary because white daylight gives a wrong value to the color scale. The reflector *S* is adjusted so as to reflect the light upward through the liquids in receptacle *G*. A tube of paper or other material, preferably black inside, about two and a half centimetres in diameter and twenty-five or thirty centimetres long, is then placed upright over the receptacle *G*, to exclude extraneous light during the examination. The eye is then applied to the upper end of the tube; on looking down this there is seen a definite dilution of blood, pale red in color, in compartment *a*, while through compartment *a'* is seen a portion of the red color scale *KK*, both illuminated by the light entering

from below. On moving the scale by means of the disc T, the color seen through compartment *a'* changes in its intensity. The scale should be moved back and forth until the color in the two compartments is made to match exactly. Then the reading of the graduated scale PP at the line M represents the percentage of hæmoglobin present, the normal amount of hæmoglobin being taken as 100 per cent.

This method is only approximate, as an exact matching of the tints is difficult, and the eyes of different individuals, or even the two eyes of the same individual, will vary in their judgment of colors. In order to promote accuracy in the matching of the colors the eye should be used only for a few seconds at a time, and then rested, in order to avoid fatigue; it is said also to be better while using the instrument to face one end of the sliding scale than to face the light, as the two lateral halves of the eye are more sensitive to color differences than the upper and lower halves. The paler tints are especially difficult to match closely; so that when the amount of hæmoglobin is very low it is better to use two capillary tubes of blood in making the dilution, rather than one, and divide the result by two. The method is probably practically correct in its findings within about five per cent, and is sufficiently close to afford clinical information of great value. A possible defect is that the readings of the Fleisch apparatus are rather low, as often blood from normal and robust individuals registers several points below 100 per cent.

As the proportion of hæmoglobin in most conditions is supposed to vary closely according to the specific gravity of the blood, tables such as the following have been prepared showing the approximate percentage of hæmoglobin corresponding to the various specific gravities (Hammerschlag's method).

Specific gravity.	Hæmoglobin.	Specific gravity.	Hæmoglobin.
1033-1035	25-30 per cent.	1048-1050	55-65 per cent.
1035-1038	30-35 "	1050-1053	65-70 "
1038-1040	35-40 "	1053-1055	70-75 "
1040-1045	40-45 "	1055-1057	75-85 "
1045-1048	45-55 "	1057-1060	85-95 "

The specific gravity being determined, this table shows the corresponding percentage of hæmoglobin; but these figures are not applicable in cases of dropsy, leukæmia, and perhaps certain anæmias.

Enumeration of the Red Corpuscles.—The number of red and white corpuscles to the cubic millimetre of blood is determined

by means of the Thoma-Zeiss hæmacytometer (Fig. 10), an apparatus which yields very satisfactory results, reliable probably within two or three per cent. This outfit consists of (a) two pipettes for diluting the blood to a definite degree, one for the red and one for the white corpuscles, and (b) a glass slide with a ruled chamber for counting the corpuscles under the microscope.

In counting the red corpuscles it is first necessary to dilute the blood to a sufficient and known degree (one or two hundred



FIG. 10.—Thoma-Zeiss Haemacytometer. (Leitz.)

times) to bring the corpuscles within countable numbers. For this purpose a number of dilution fluids are available, about the best of which is that of Gowers, the formula for which is as follows:

Sodium sulphate.....	7.5
Acetic acid.....	20.
Water, distilled.....	125.

This and all other diluting fluids should be kept perfectly clear and free from solid particles by filtering frequently, or whenever sediment collects from long standing; the presence of foreign particles seriously interferes with the process of counting the corpuscles. Another diluting fluid for the red corpuscles is that of Toisson, which stains the leucocytes violet, and is therefore especially useful when the leucocytes are relatively numerous and it is convenient to have them made conspicuous so as to

omit them in counting. The composition of Toisson's fluid is as follows:

Methyl violet 5 B.....	0.025
Sodium chloride	1.
Sodium sulphate.....	8.
Neutral glycerin.....	30.
Water	160.

For counting the red corpuscles the blood is measured and diluted by a special pipette for these corpuscles (Fig. 11). This has a bulbous expansion or chamber (E) in the course of a capillary tube. The longest portion of the capillary tube is graduated into ten equal parts from the point (S) up to the mark 1.

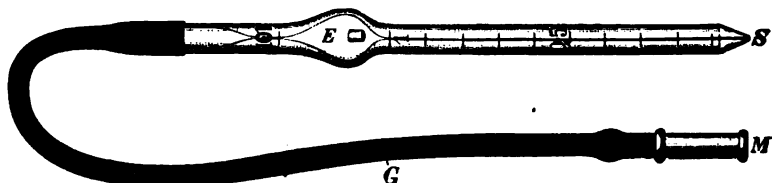


FIG. 11.—Pipette for Diluting Red Blood Corpuscles. (Spencer Lens Company.)

The bulbous portion is of such size that from the mark 1 to mark 101 its capacity is exactly 100 times that of the ten divisions of the capillary tube. The bulb contains a small piece of glass to aid in mixing the blood. To the blunt end of the pipette can be attached a rubber tube (G), provided with a mouthpiece M.

To use the pipette, the mouthpiece is taken in the lips or teeth, the point of the tube is immersed in the drop of blood flowing from the ear, and by gentle suction blood in a continuous and unbroken column is drawn into the capillary tube exactly to one of the divisions. Superfluous blood being removed from the outside, the point of the pipette is then quickly plunged into the diluting fluid, which is drawn up into the instrument exactly to mark 101. Twirling the tube while drawing in the dilution fluid aids in breaking up clots and mixing the corpuscles. With the blood drawn up to mark 1, and the diluting fluid to mark 101, the blood is diluted exactly 100 times. The blood may be drawn to any of the divisions of the capillary tube, not necessarily to mark 1, but the corresponding dilution must be noted to be used in the subsequent calculations. Usually it is most convenient for counting to draw the blood to mark 0.5, which

gives a dilution of .5 to 100, or 1 to 200. After filling the pipette, the rubber tube is removed, the two ends of the pipette are closed by the thumb and a finger, and the pipette is shaken vigorously a few times to mix the corpuscles thoroughly with the diluting fluid; the pipette may be also twirled for the same purpose. The pipette is then laid aside in a horizontal position until the count is made; or if it is to be transported some distance it may be placed lengthwise in a rubber band which tightly closes the two ends.

The pipette must be perfectly clean and dry at the time of use. No time should be lost in the manipulations, otherwise the

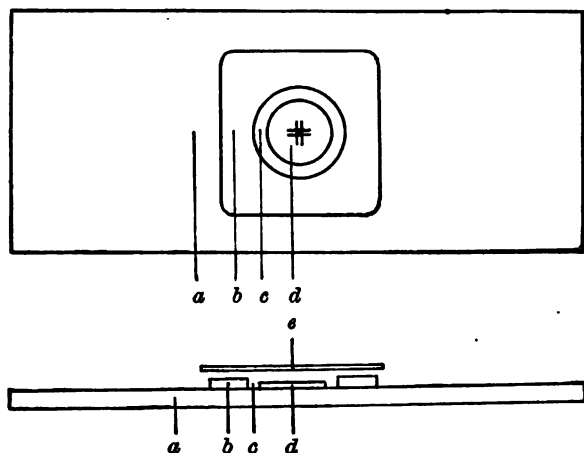


FIG. 12.—Ruled Slide and Chamber for Counting Blood Corpuscles, top and side plans.

blood will clot in the tube and cause trouble, vexation, and delay in cleaning. If the blood is once drawn up above the mark at which the dilution is to be made, the pipette must be cleaned and dried before further use, as the corpuscles adhering to the tube will falsify the result.

The next step is counting the corpuscles, which is done by means of a ruled counting chamber (Fig. 12). This consists of a glass slide (a) to which is cemented a square glass plate (b) with its center cut out in circular form. In the middle of this circular opening is cemented to the slide a smaller glass disc (d) precisely one-tenth of a millimetre less in thickness than the outer glass slip. A thick, perfectly plane cover-glass (e) rests upon the slip b. The middle of the central glass disc (d) is

ruled into two sets, at right angles to each other, of twenty-one parallel lines exactly one-twentieth of a millimetre apart, thus forming four hundred small squares (twenty squares each way), the area of each of which is $\frac{1}{400}$ of a square millimetre; through every fifth row an extra line is ruled, as a guide to the eye. When the cover-glass rests upon the slip *b*, in perfect contact with it, the space inclosed in each small square is exactly $\frac{1}{40000}$ cubic millimetre in volume.

When the corpuscles are to be enumerated, the counting chamber and cover-glass are thoroughly cleaned and freed from dust particles. The pipette being shaken and twirled to diffuse the corpuscles evenly through the fluid, the fluid is gently blown out of the pipette, and, the first few drops being thrown away, a drop of the mixture is placed on the ruled disc of the counting chamber. The cover-glass is then carefully applied over it. The drop should be of just sufficient size completely or nearly to cover the disc *d* when the cover slip is adjusted, without running over into the surrounding moat (*c*); bubbles or particles should be excluded. The cover-glass should rest in perfect contact on the glass slip *b*, which cannot be brought about unless the two surfaces are perfectly clean; contact may be known to be complete when a play of colors (Newton's interference rings) is permanently visible at some point under the cover-glass.

The chamber being thus prepared, it is focussed under the microscope, using a low-power objective (about 2 centimetres) and a strong ocular (or with the tube lengthened), with a dim substage illumination. With this magnification the four hundred squares just about occupy the entire field, and the count can be made without moving the slide. Thickly and evenly scattered over the field will be seen the red corpuscles with occasional leucocytes, with the ruled lines underlying them visible. Should the ruling not be distinct enough, the lines can have been darkened by rubbing into them some fine soot (obtained by holding a piece of cold glass in a match flame).

The corpuscles in a given number (from one hundred to three hundred) of squares are then to be counted. In counting over the squares some special order should be followed; thus, the corpuscles may be counted in straight rows of squares, twenty squares in each row, across the field; or the sets of squares (sixteen to thirty-six squares in each set) marked off by the double ruling

may be counted separately. Many of the corpuscles will be found lying directly on the lines, and the question arises whether to regard them as in or out of the square being counted; in such cases, all the corpuscles lying on one of the vertical and one of the horizontal sides of the squares should be uniformly included in the count, while those lying on the other vertical and the other horizontal side are excluded. Thus, all corpuscles on the upper and left-hand lines of each square may be counted in, while all on the lower and right-hand lines are excluded. In this manner the doubtful corpuscles are equalized and this source of error is eliminated.

The actual count being completed, the determination of the number of the corpuscles to the cubic millimetre of blood is made by a simple arithmetical calculation, based on (*a*) the number of corpuscles counted in (*b*) a definite number of squares, (*c*) the volume corresponding to each square ($\frac{1}{1000}$ cubic millimetre), and (*d*) the extent to which the blood was diluted. The rule is, multiply the average number of corpuscles in each square by 4,000, and this again by the amount of dilution. Or, algebraically, the number of erythrocytes per cubic millimetre = $\frac{4,000 \text{ } ad}{b}$. Thus, if in 160 squares 996 corpuscles are counted, the dilution being 0.6 to 100, the calculation is as follows:

$$\frac{996 \times 4,000 \times 100}{160 \times .6} = 4,150,000 \text{ red corpuscles per cubic millimetre.}$$

From the number of red corpuscles, the percentage to the normal number (5,000,000) can be readily calculated (thus, 4,150,000 = 83 per cent of normal).

After the count is concluded the apparatus should be carefully cleaned. The ruled slide and cover-glass are simply rinsed with water (avoiding the use of alcohol or ether) and dried. The contents of the pipette are blown out; clean water is then drawn into it, and expelled again, one or more times till its interior is well rinsed; lastly, the pipette is filled with a mixture of equal parts of alcohol and ether, again emptied, and air drawn through it until its interior is dry, which is shown by the glass ball inside the bulb not adhering to the wall. If the inside of the pipette is much smeared or stained or contains particles, it may be left filled with a cleansing fluid (such as the

bichromate fluid mentioned on page 5) for several hours. When the blood clots in the capillary tube, it may form an obstruction impossible to blow out; in this case it, or any similar obstruction, may be dislodged by introducing a fine brass or copper wire, such as is used for hypodermic needles, after which acids or other cleansing fluids may be left in until all traces of foreign matter are destroyed. Brass or copper wire should be used in this manœuvre, so that if a portion of the wire should break off and be left in the tube it can be dissolved out again by introducing nitric acid. Nothing of a pointed or conical shape should be inserted into the tube for cleaning purposes, as the wedge action is very apt to break a chip from the point of the tube.

The use of the centrifugal apparatus or hæmatokrit as a means of indirectly estimating the number of red corpuscles was once advocated, but it is now little used for the purpose, being no more accurate and no more convenient than the Thoma-Zeiss method. Each one hundredth of volume of the red corpuscles as determined by this instrument corresponds roughly to 100,000 corpuscles per cubic millimetre; thus, the hæmatokrit shows the volume of red corpuscles in normal blood to be about 50 per cent, which corresponds to 5,000,000 per cubic millimetre, or about 100,000 to each unit of per cent. The size of the corpuscles, however, as well as their number, is a factor in the total volume.

Determination of Corpuscular Hæmoglobin-Ratio.—The richness or index of the individual red blood corpuscles in hæmoglobin (see page 20) is obtained by dividing the percentage of hæmoglobin, as determined by the Fleischl instrument, by the percentage of the red corpuscles to the normal number. Thus, if the hæmoglobin is 63 per cent, and the number of red corpuscles is 3,750,000 per cubic millimetre (or 75 per cent of the normal number), the corpuscular hæmoglobin-ratio is $\frac{63}{75}$, or .84.

Enumeration of Leucocytes.—The leucocytes are counted in the same way as the red corpuscles, except that the blood must be diluted to a less degree (requiring a special pipette), different diluting fluids are necessary, and a greater number of squares must be counted. The pipette for the leucocytes is similar to that for the red cells, but is adjusted for a maximum dilution of 1 to 10, with graduations permitting higher dilutions up to 1 to 100; the mark above the bulb is 11 instead of 101 as in the red pipette. The calibre of the capillary tube of the leucocyte pipette is larger than that of the red pipette, requiring a greater

amount of blood to fill it, which makes this pipette more difficult to manipulate than the other. The diluting fluid used is a .33 to .50 per cent solution of glacial acetic acid in water, kept filtered clear. This fluid dissolves out the red corpuscles, leaving the leucocytes alone, clear and refractile. When filled the leucocyte pipette should be kept in a horizontal position or with the ends closed by a rubber band, in order to prevent its contents escaping, which occurs very readily through the large bore.

The same counting chamber is used as for the red corpuscles, but owing to the less number of leucocytes it is necessary to count a much greater number of squares. A number of fields must be prepared, and each time the leucocytes in the entire four hundred squares counted. The manner of calculation is the same as for the erythrocytes; thus, if in 1,600 squares 156 leucocytes are counted, the dilution being 1 to 20, the number of leucocytes is $156 \times 4,000 \times 20 \div 1,600$, or 7,800 per cubic millimetre.

In cases where there is an enormous increase in the leucocytes it may be most convenient to use the red pipette, with a higher dilution than the leucocyte pipette affords. Except in the points mentioned the technique of counting the white corpuscles is identical with that for the erythrocytes.

Determination of Proportion of White to Red Corpuscles.—The number of red corpuscles for each leucocyte is determined by dividing the number of red corpuscles per cubic millimetre by the number of white corpuscles in the same volume.

Microscopical Examination of Fresh Blood.—This procedure involves very little trouble, and is capable of affording very valuable information. To prepare the specimens a clean cover-glass (No. 1 in thickness), held in forceps or between the thumb and forefinger, is touched to the blood oozing from the puncture so that a small drop of blood adheres to the middle of the cover-glass. This is then laid on a clean glass slide, and the blood is allowed to spread out in a thin layer between the cover and slide. The blood drop must be very small; if too large the layer of blood is too thick for proper examination; practice soon teaches the proper size of drop to be taken. If the glass is clean and free from oil, the blood spreads out readily; if necessary, the cover may be gently pressed down with the point of the forceps to assist the spreading of the blood. If it is examined immedi-

ately, no further treatment is necessary. But if the specimen is to be kept some time before placing it under the microscope, by means of a small camel's-hair brush a ring of vaseline should be applied around the edge of the cover-glass so as to exclude air and prevent evaporation of the blood plasma; by thus sealing the specimen, it may be kept for hours without change. The specimen is examined under the microscope with the oil-immersion objective.

Examination of fresh blood in this manner reveals abnormalities in the size, shape, and nucleation of the red cells; shows rouleau formation; enables some judgment to be formed as to the amount of hæmoglobin in the individual corpuscles, from the intensity of their color or the pallor of their centres; shows the presence of granules, pigment, fat, malarial or other parasites, and occasionally bacteria; exhibits to a certain degree the rapidity and amount of fibrin formation; and permits a rough idea to be formed as to any marked increase in the proportion of the leucocytes.

Malarial parasites are better searched for in fresh than in stained blood, as with their pigment granules, refractile protoplasm, and amœboid movements they are quite conspicuous objects. In case they are not at first found it is necessary to search over a considerable quantity of blood, or a number of preparations, before a negative report can be made. Systematic search is greatly facilitated by the use of a mechanical stage. Prolonged search is also necessary for the filaria, but in this case lower powers may be used.

Examination of Stained Specimens.—For the demonstration of certain features or the preservation of permanent specimens it is necessary to fix and stain the blood. Three points in the technique require special consideration, preparation of the dried blood film, fixing, and staining.

To make the *cover-glass preparations* of dried blood, a small drop of blood, of just the right size (to be learned by practice), is obtained on the middle of a clean cover-glass, as in preparing a specimen of fresh blood; this is then laid on another cover-glass, held near its edge in the grasp of a self-retaining forceps (such as Stewart's, Fig. 13), so that the blood spreads out between the two cover-glasses in a thin, even layer. If necessary, the spreading of the blood can be facilitated by gently pressing

the two glasses together between the points of thumb forceps. When fully spread, the edge of the first cover is grasped by forceps and the two glass slips are separated by sliding them apart, leaving a thin, even film of blood on each. The cover slips are then allowed to dry in the air, in which condition they may be kept indefinitely before further treatment. According to some

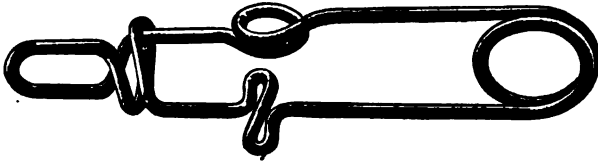


FIG. 13.—Stewart's Self-retaining Cover-glass Forceps. (Spencer Lens Company.)

authorities, while preparing the spreads the covers should be held in forceps and not in the fingers, the heat of which may cause deleterious changes in the corpuscles. A considerable proportion of cover-glass spreads of blood made in this way will be failures, not presenting satisfactory microscopical pictures; consequently it is necessary to prepare a proper number of cover-glasses, so that from among them a sufficient number of good specimens may be available.

For the filaria, a considerable amount of blood may be spread over a slide, dried, and laid away for future staining or examination.

Before staining, *fixation* of the blood films is necessary, for which a number of procedures are available:

a. Fixation may be accomplished by exposure to a temperature of 110° to 120° C., for ten to twenty minutes. This may be managed by laying a sheet of copper about 40 centimetres long and 10 or 15 centimetres wide on a laboratory iron tripod, and standing a Bunsen burner underneath so that its flame strikes one corner of the copper sheet. When the sheet becomes fully heated, drops of water are placed upon it at different distances from the flame in order to determine where it just boils, thus locating the point at which a constant temperature of 100° is maintained. A short distance nearer the flame will give the approximately proper temperature for fixing the blood. The cover-glasses are laid on the copper at this point and allowed to remain ten to twenty minutes. Blood smears may also be tolerably well

fixed, and more quickly, by passing them through the Bunsen flame ten to twenty times at a rate to be learned by practice and test of the specimens.

b. The blood spreads may be fixed by immersion in absolute alcohol ten to thirty minutes.

c. By immersion in a mixture of equal parts of absolute alcohol and ether two hours.

d. By immersion in a 5 per cent solution of mercury bichloride in alcohol for several hours, or over night, followed by thorough washing and drying.

e. A rapid method of fixation by immersing the spreads for one minute in a 1 per cent solution of formalin (.4 per cent formaldehyde) in alcohol has been recommended.

Of these methods of fixation that by heat is most used for staining with the triple stain, although the alcoholic methods often give good results, especially for red corpuscles and the malarial parasite.

After fixation many methods of *staining* the blood are available for various purposes, but the stains ordinarily most useful are eosin and methylene blue, the triple stain, and basic stains. Blood is very uncertain in its staining reactions, some specimens staining nicely, others for unaccountable reasons staining very poorly.

Eosin and Methylene Blue Method.—A number of methods of staining blood with these stains are available. One of the easiest and most satisfactory is as follows: After fixation with alcohol or otherwise, stain in saturated aqueous solution of eosin fifteen to thirty seconds; wash in water; stain in 2 per cent aqueous solution of methylene blue one or two minutes, or until sufficiently stained; wash, dry, and mount in balsam. This method is capable of giving excellent results, but variable effects are caused by slight variations in the composition of the dyes used. Methylene blue as sold is especially variable, sometimes the best makes being so powerful that they wash out the eosin; but with a lot that works well with the eosin good results are obtainable.

Another method considerably employed is that of Chenzinsky, whose solution is as follows:

Methylene blue, saturated aqueous solution.....	40 c.c.
Eosin, .5 per cent solution in 70 per cent alcohol.....	20 "
Water, distilled.....	40 "

The specimens, slightly fixed, are left in this solution, which should be filtered just before using, six to twenty-four hours at a temperature of 37° C. This solution stains well if the ingredients are in just the right proportions; changes may be made, if required, until a satisfactory mixture is obtained.

In blood stained with eosin and methylene blue the red corpuscles are stained red, the nuclei of the leucocytes and nucleated red corpuscles blue, oxyphile or eosinophile granules a brilliant red, neutrophile granules pinkish, malarial parasites pale blue, basophile granules blue. The method is excellent for showing malarial parasites and nuclei, but is not satisfactory for differentiating the leucocytes.

Triple Stain.—This stain (also known as the triacid stain, Ehrlich's stain, Biondi's stain, Heidenhain's stain) is the one in most general use in hæmatological work. Fixation is usually done by heat, but the alcoholic solution of mercury bichloride often gives as good results. Insufficient heating causes this stain to act too deeply, excessive heating makes it stain too feebly. Like other methods of staining the blood the triple stain is somewhat uncertain. It is very difficult to prepare solutions of the stain that will work satisfactorily, as even with the most reliable makes of the ingredients different solutions prepared in the same way may yield very different results.

To prepare the stain, separate saturated aqueous solutions are made of orange G, acid fuchsin, and methyl green (all of the best quality, as Grüber's), and allowed to stand several days. Orange G is not very freely soluble in water; acid fuchsin and methyl green are very soluble, especially the former, and the amount of water and stain used should be judged accordingly. After the solutions have stood a long enough time the staining fluid is prepared approximately as follows:

Orange G, clear saturated solution	6. c.c.
Acid fuchsin, clear saturated solution.....	4. "
Methyl green, clear saturated solution, to be added drop by drop, shaking the mixture at the same time	6.6 "
Glycerin	5. "
Absolute alcohol	10. "
Water	15. "

The amounts given are approximate, and if on trial the mixture does not stain properly, additions should be made of the

ingredients defective, or the proportions should be modified until a satisfactory solution is obtained. The fluid keeps indefinitely.

Owing to the difficulty and uncertainty of preparing the solution, ready mixed powders and solutions are on the market, but they are usually as uncertain as the fluid prepared from the original ingredients.

The method of staining is to apply a few drops of the solution to the blood specimen, properly fixed, for from two to six minutes; wash with water, dry, and mount in balsam.

By this method, when it acts properly, the red corpuscles are stained orange (not red); polychromatophile red corpuscles stain brownish, or blackish, or other abnormal colors; the nuclei of the leucocytes and nucleated red corpuscles stain from light to deep green or blue; neutrophile granules stain violet or reddish; eosinophile granules stain a brilliant red; malarial parasites stand out unstained; basophile granules are not brought out at all.

Examination of specimens stained in this way reveals abnormalities of the red corpuscles as to size, shape, or poikilocytosis, polychromatophilia, and the presence of nuclei; from the intensity with which the red corpuscles are stained or the pallor of their centres the amount of hæmoglobin can be roughly estimated; the different varieties of leucocytes can be distinguished and their relative numbers ascertained by differential enumeration; and the relative number of nucleated red corpuscles and other objects may be determined by indirect enumeration.

Differential Enumeration of Leucocytes.—The relative proportions or numbers of each of the different varieties of leucocytes are determined by going systematically over specimens stained by the triple stain and counting all the different kinds of leucocytes encountered. A mechanical stage is of great assistance in this work, but not indispensable. At least several hundred or a thousand leucocytes should be counted, and the results reduced to percentages.

Thus, if in a differential count of 850 leucocytes, 221 are small mononuclears, 34 large mononuclears, 578 polynuclears, and 17 eosinophiles, the percentages of each (found by dividing by 8.50) are small mononuclears 26, large mononuclears 4, polynuclears 68, and eosinophiles 2 per cent.

The total number of leucocytes per cubic millimetre and the

relative percentage of each variety being known, the number of each variety of leucocyte to the cubic millimetre can be readily calculated, and these numbers compared with the normal numbers of each will show in what varieties there has been any real change in number.

Enumeration of Nucleated Red Corpuscles.—The number of nucleated red corpuscles relatively to the total number of erythrocytes, or their number per cubic millimetre, may be determined by counting the normoblasts and megaloblasts along with the leucocytes in the differential count; this gives the ratio of the number of nucleated red cells to the total number of leucocytes, from which, and the number of leucocytes per cubic millimetre, or the proportion of white to red corpuscles determined by counts of those cells, the numerical proportions of the nucleated erythrocytes can be determined.

Thus, supposing that in a differential count m megaloblasts and n normoblasts are seen along with o leucocytes, and that the leucocytes by count number c to the cubic millimetre of blood, the number of megaloblasts and normoblasts per cubic millimetre would be $\frac{cm}{o}$ and $\frac{cn}{o}$ respectively. Or, if the numerical proportion of the white to the red corpuscles is 1 to r , the proportion of the megaloblasts to the total number of red cells would be 1 to $\frac{or}{m}$, while there would be 1 normoblast in every $\frac{or}{n}$ red cells.

This is the method of **indirect enumeration**, and may be applied to the enumeration of other bodies, as blood plates and malarial or other parasites, the numerical ratios being established by comparing the counted number of leucocytes with that of the bodies to be enumerated.

Basophile Stains.—For demonstrating the basophile granules of leucocytes, the specimens may be stained by one of the following methods:

a. Stain in saturated aqueous solution of methylene blue for two to ten minutes; this stains basophile granules and nuclei blue.

b. Stain for several hours in the following:

Glacial acetic acid	12.5
Absolute alcohol.....	50.
Water	100.
Dahlia nearly to saturation.	

After staining, wash in water, and remove excess of stain with alcohol. This stains the basophile granules reddish-violet or violet, nuclei violet.

c. Stain the specimens, preferably fixed with mercury bichloride, for several minutes in the following:

Toluidin blue	1
Phenol.....	5
Water	95

After staining, treat with alcohol, or 95 or 70 per cent alcohol to which 1 per cent of hydrochloric acid has been added, until sufficiently differentiated. This stains nuclei and basophile granules blue or (in the case of mast-cell granules) reddish.

Widal Test for Typhoid Fever.—This reaction is based on the fact that in typhoid infections a substance (an agglutinin) is generated in the blood which, when added to young typhoid bacilli, causes the germs to mass together in clumps and lose their motility. Sometimes blood from healthy individuals or in other diseases than typhoid produces this effect on typhoid bacilli, but never so powerfully as the blood of typhoid patients. If diluted sufficiently, only the blood of those ill with typhoid will give the reaction within certain limits of time. Consequently, specimens of blood which under the proper conditions of dilution and time cause agglutination and arrest of motility of typhoid bacilli must have come from persons who were or at some previous time had been suffering from typhoid fever; and this affords a test for typhoid fever which is very extensively used.

In practice, much depends on the culture of typhoid bacilli. Different cultures of this bacillus vary exceedingly in their reaction with typhoid blood, some cultures exhibiting it very markedly, while others hardly respond at all. Bacilli that are very motile in 24-hour cultures usually react well. A culture that gives the Widal reaction well should be obtained for this purpose, stock cultures on agar being transplanted about once a month. For the actual test, cultures from 18 to 24 hours old made from the stock are necessary, as only while young are the bacilli sufficiently motile. The culture medium may be either bouillon or agar. Bouillon is the medium generally specified, a two-per-cent glucose peptone bouillon, neutral or very slightly acid, giving the best growth. It is often quite difficult to prepare a bouillon in which a sufficiently abundant growth of the

typhoid bacillus will take place within 24 hours. Ordinary agar is much more certain in its action, and gives an ample growth of very active bacilli, and should afford as good results as bouillon cultures. The motility of the bacilli is greater the lower the temperature at which they are grown; the cultures should be kept, therefore, at the lowest temperature at which a sufficiently profuse growth can be obtained within the requisite time, that is, at room temperature rather than in the oven if the lower temperature gives sufficient growth.

The specimen of blood may be taken in three ways, the fresh blood, the separated serum, or dried blood being used for the test. The serum gives the best results, but is more difficult to prepare; the dried-blood method is very convenient and simple of management, and although perhaps less potent and less accurate as far as dilution goes, is the most commonly used.

To obtain the fluid serum a capillary glass tube is prepared in the Bunsen flame, and into this a column of blood is drawn by gentle suction. The capillary tube is then sealed by heat at both ends, leaving the blood inclosed, and is immediately revolved rapidly in the centrifugal machine. This throws the corpuscles down and leaves a column of clear serum above. In this form the serum may be kept indefinitely. When it is to be used, a file scratch is made at the junction of the corpuscles and serum, the tube is broken at that point, and the serum is expelled.

In the dried-blood method, three or four small drops of blood are placed separately on a glass slide or piece of paper and allowed to dry on it. Thus prepared the blood will keep for days or weeks, until the test is made. One of the objections to this method is that it does not permit accurate dilutions to be made. To obviate this, various more or less complicated methods for making the blood drops of known and definite size have been proposed; the best for practical purposes is to take the blood drop with the same bacteriological platinum loop that is used subsequently to make the dilution. As the hæmatologist has, however, to make tests with specimens that have been prepared by practitioners in general without special care, he is compelled largely to learn to make his dilutions approximately correct according to his best judgment.

Observers have not yet come to final agreement as to the amount of dilution or the length of time required to exclude

pseudo-reactions in non-typhoid cases. The present tendency is to the use of high dilutions, even up to 1 to 200. Non-typhoid blood will probably only very rarely give pseudo-reactions with dilutions of 1 to 20 or 30 within a limit of one hour, or with dilutions of 1 to 40 or 50 within two hours; and these dilutions and time limits may be adopted for practical purposes.

To make the test, a cover-glass and a slide with a concavity in its centre are cleaned. Vaseline is applied with a small camel's-hair brush around the margin of the concavity.

If dried blood is used, by means of the platinum loop sterile distilled water in sufficient amount to make the proper dilution is added to the dried blood spot on the glass slide and rubbed up with it so as to redissolve the constituents of the serum; if the dried blood is on paper, cut out the blood spot and rub it up with water in a watch-glass. If agar cultures are employed, a drop or loop of water is placed on the centre of the cover-glass, and with it is mixed a sufficient quantity of bacilli removed from the agar on the point of the platinum needle. To this is added

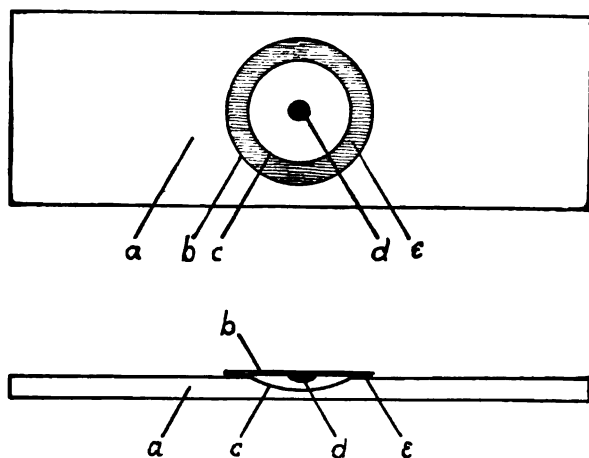


FIG. 14.—Arrangement of Hanging Drop for Widal Test and Examination of Bacteria in General, top and sectional view. *a*, Slide; *b*, cover-glass; *c*, concavity in slide; *d*, hanging drop; *e*, ring of vaseline.

a sufficient amount of diluted serum prepared from the dried blood, and the whole well mixed. The degree of dilution is gauged by the number of loops or drops of water added. The cover-glass with the drop in its centre is then inverted and ap-

plied over the concavity in the slide, so that the drop hangs beneath the cover in the concavity, while the vaseline holds the cover-glass in place, seals its edges, and prevents evaporation.

If, with dried blood, bouillon cultures are used, the dried blood is rubbed up with a little water and the dilution completed to the proper degree with the bouillon culture; with a drop of

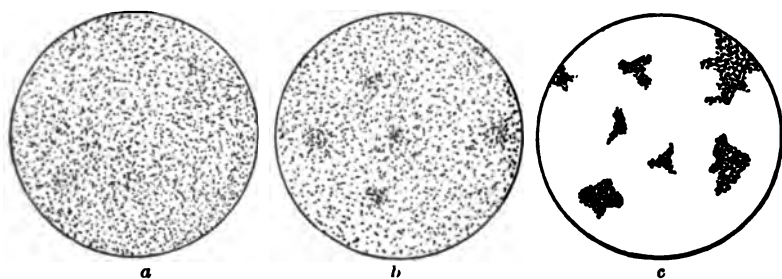


FIG. 15.—Widal Test for Typhoid Fever. *a*, Negative reaction; *b*, partial reaction; *c*, positive reaction.

this mixture a hanging drop is arranged as just described. If fluid serum or fresh blood is used for the test, the dilution may be most accurately made by measuring off the proper amounts of serum and bouillon or water with a graduated capillary tube; the hanging drop is prepared as with the dried blood.

The hanging drop thus prepared is examined with a dry lens of about 4 millimetres focal length, the illumination being dim. When properly prepared, the bacilli should be pretty closely crowded together, and yet not so densely packed together as to interfere with their movements. If the Widal reaction is absent (or negative), the bacilli exhibit the same appearance as in an ordinary culture; they are scattered evenly over the field, all exhibiting Brownian movements and many moving actively about with tumbling or wriggling movements. If the Widal reaction is present (or positive), the bacilli become motionless, and instead of being uniformly distributed become aggregated or agglutinated together in irregular, densely packed clumps or masses of variable but comparatively large size; the spaces between the clumps are clear and void of bacilli. The positive reaction may appear instantly, or it may develop slowly; but if it does not appear within the proper time limit the result is to be regarded as negative. Sometimes a condition, the "partial reaction," is presented intermediate between the negative and the positive

reaction, in which the bacilli show some tendency to collect in small clumps, but without the intervening spaces becoming clear. The result of the test may therefore be either negative, partial, or positive; in conjunction with the result it is well to make a record of the dilution employed and the time required for positive reactions to develop (or the time limit in cases of negative results).

Probably upward of 95 per cent of all cases of typhoid fever give the Widal reaction at some time in their course, usually within the first seven or ten days. Exceptionally, the reaction is absent throughout, and not infrequently it appears so late that its diagnostic value is greatly impaired. The reaction frequently persists for months and years after an attack of typhoid fever. On the other hand, with sufficiently high dilution and corresponding time limit, the reaction practically never appears in connection with any other conditions than typhoid fever.

A positive reaction, with proper conditions of dilution and time, may therefore be accepted as a pretty conclusive indication that the patient furnishing the blood has, or has had, typhoid fever. The higher the dilution and the shorter the time giving a positive reaction, the stronger is the proof. On the contrary, a negative reaction, even if repeatedly found, does not prove that the disease is not typhoid fever; still, negative results throughout a case have sufficient negative significance to warrant careful consideration of a diagnosis of typhoid. On the whole, the Widal test is of considerable diagnostic value; but its limitations must be recognized and it must be properly interpreted.

The reaction is sometimes given by the urine or other fluids of affected persons. The test can be reversed, and known agglutinating typhoid blood may be used to test whether a suspected bacillus is the typhoid bacillus or not. The agglutination test is also applicable to a few other infectious diseases, in connection with their specific micro-organisms, as cholera, bubonic plague, Malta fever.

Determination of Volume of Red Corpuscles.—The volume of the red corpuscles relatively to that of the entire blood is determined by the centrifugal method. The centrifuge attachment used for blood work, the "hæmatokrit" (Fig. 1), consists of a horizontal arm into each end of which can be firmly clamped a capillary tube 5 centimetres long divided into 100 equal divisions. By means of a rubber tube (such as is used with the hæmacytometer) slipped on an end of one of the capillary tubes, the tube is by suction filled

with blood; the free end of the glass tube is then closed with the finger, and the rubber tube removed. The glass tube is then clamped securely in place in the hæmatokrit arm, care being taken that its ends are tightly adjusted and closed so that the blood cannot escape during the high centrifugal pressure. Both tubes should be attached, to maintain equilibrium. The arm is then revolved at a high rate, about 10,000 revolutions per minute for two or three minutes. At the end of this time the red corpuscles will be found packed in the distal end of the tube, the plasma in the proximal end, and the proportionate space occupied by the red corpuscles can be read off from the graduated scale.

This method gives the proportionate volume of red corpuscles in normal blood as about 50 per cent, slightly in excess of the true volume. The leucocytes normally occupy a minute space between the erythrocytes and plasma, but in leucocytosis present greater volume.

This procedure is not at the present time very much employed in hæmatological work. The instrument is noisy, and inconveniently requires immediate centrifugalization of the blood before it coagulates. At one time the method was advocated as an easy method of determining the number of red corpuscles, each division or hundredth corresponding approximately to 100,000 corpuscles per cubic millimetre. What the procedure really shows is the relative volume of the red corpuscles, which varies not only with the number but with the average size of these cells. Taken in conjunction with an actual count of the red corpuscles the method affords a means of determining the average volume of the individual cells.

Calculation of the Average Volume of Individual Red Corpuscles ("Volume index").—Having determined the number of corpuscles per cubic millimetre (1 cubic millimetre=1,000,000,000 cubic micromillimetres) and the total relative volume of the corpuscles, multiply 1,000,000,000 by the volume percentage, and divide the product by the number of corpuscles per cubic millimetre; the quotient expresses the average bulk of each red corpuscle in cubic micromillimetres. Thus with the normal number of erythrocytes, 5,000,000 per cubic millimetre, and the normal volume obtained by the centrifugal machine, 50 per cent, the average volume of each red corpuscle is 100 cubic micromillimetres. The volume as thus calculated from the hæmatokrit finding is somewhat in excess of the real volume (normally about 85 cubic micromillimetres), but still would suffice for clinical purposes, 100 being a convenient standard for comparison.

Determination of Specific Gravity of Blood.—Hammerslag's method: A mixture of chloroform (whose specific gravity is 1.526) with benzin (specific gravity .889) is made in such proportions that the specific gravity of the mixture is about that of the blood. A drop of the blood free from air bubbles is allowed to fall directly into this mixture, in a cylindrical receptacle. If the blood drop then remains stationary in the middle of the mixture, without any tendency to either sink or rise, its specific gravity is the same as that of the mixture; if the blood drop sinks, it is heavier than the mixture; if it rises, lighter. In case the mixture is not of the same specific gravity as the blood, it must be made so by adding chloroform (if the blood sinks) or benzin (if the blood rises), mixing the fluids thoroughly after each

addition. When the chloroform-benzin mixture is brought to the specific gravity of the blood, so that the drop neither rises nor sinks, its specific gravity is taken with an ordinary urinometer; the reading gives also the specific gravity of the blood. The chloroform-benzin mixture may be filtered and preserved.

A more accurate method, that can be employed if delicate balances are available, is to weigh a special capillary tube of definite capacity filled with the blood and again when filled with pure water; from which the specific gravity can be determined.

Bacteriological Examination of Blood.—Bacteria in the blood, even when causing serious symptoms, are usually so relatively few and scattered that to make an adequate search for them it is necessary to use several cubic centimetres of blood. This is taken, under strict asepsis, by introducing the needle of a hypodermic syringe into a vein of the arm or elsewhere and filling the syringe with blood. The blood is then discharged over the surface of suitable culture media (as blood serum or agar) and any resulting growth investigated by the usual bacteriological methods.

Chemical Examination of Blood.—If practicable methods were available for investigating chemically the constituents of the blood, especially for determining as to the presence and nature of toxins or other poisonous substances, much information of clinical value could thereby be elicited. There are numerous tests and estimations that can be made if large enough quantities of blood are used, such as are conducted in physiological investigations; but practically the clinical hæmatologist is ordinarily limited to such tests as he can make with minute amounts of blood.

The estimation of hæmoglobin and the Widal test for the typhoid agglutinin are really of chemical nature; and a few other procedures are available that are of occasional service, such as the following:

Total Solids.—A few drops of blood are collected in a watch-glass, which is covered with another to prevent evaporation, and the whole weighed with delicate scales. The specimen, uncovered, is dried for twenty-four hours at 60° or 70° C., and again weighed. From these data, and the weight of the clean watch-glasses, the weight of the solids and the water in the blood can be determined.

Hæmoglobinæmia.—By the centrifuge and hæmatokrit tube throw down the corpuscles in a small amount of blood. Normally the upper column of serum is clear and colorless, but in hæmoglobinæmia it will be tinged with red from the hæmoglobin abstracted from the corpuscles.

Fat.—The presence of particles of fat is best tested for by the Sudan III. method (page 10).

Glycogen is detected by its turning brown or mahogany color with iodine. Thin cover-glass spreads of blood, without fixing, are mounted in a drop of the following solution placed on a slide:

Iodine	1
Potassium iodide	8
Water	100
Gum acacia sufficient to make syrupy consistency.	

A better method is said to be to place the cover-glass films for a few minutes in a small jar containing a few iodine crystals, and, when sufficiently stained, to mount them on a slide in a saturated solution of levulose.

On examination with the oil-immersion lens glycogen granules are found stained deep brown, other elements being lemon yellow. Increase of the glycogen in the plasma or leucocytes or a general brown coloration of the leucocytes is supposed to be indicative of suppuration or certain other conditions.

Diabetic Tests.—The blood in cases of diabetes mellitus exhibits peculiar reactions with staining reagents that may at times be of diagnostic use.

Bremer's test is based on the fact that the red corpuscles of diabetic blood lack the affinity they normally possess for acid stains, like eosin, Congo red, and others, while they take some stains, as Biebrich scarlet, that do not color normal blood.

Williamson's method depends on the fact that diabetic blood added to a methylene-blue solution changes the color of the latter to a yellow, while normal blood does not affect it.

The amount of *iron* in the blood can be determined by the method and apparatus of Jolles, which is not in common use. The amount of iron thus determined bears no constant ratio to the amount of hæmoglobin. Tests for numerous other constituents and methods for determining the degree of *alkalinity* of the blood have been employed, especially in physiological investigations, but on account of their complexity, the amount of blood required, or the lack of clinical significance are not used in practical medicine. The *spectroscope* is a useful instrument for investigating the blood pigments, but is rarely used for clinical purposes. The *tonicity* of the red corpuscles, that is, changes in their ability to resist the destructive influences of variations in the density and composition of the medium in which they are suspended, has been investigated to some extent, but is not yet made a matter of practical importance.

Micrometry.—In making actual measurements of the blood corpuscles the ordinary methods of micrometry may be employed.

Determination of Coagulation Time.—The time required for coagulation may be sometimes desired, as in connection with hæmorrhagic or hæmophilic conditions.

One method that has been recommended consists in introducing into a capillary tube, 3 to 5 centimetres long and having a calibre of about a millimetre, a column of blood about $\frac{1}{4}$ centimetre long, and passing through this a white horsehair about 10 centimetres long, cleaned and freed from fat by alcohol and ether. Until the blood clots the horsehair on being removed from the blood remains unstained; as soon as clotting begins the horsehair on removal is stained red. The hair, held by its end, is pushed onward through the blood about half a centimetre every half-minute or so until clotting is seen to occur.

The tube should be so held that the heat of the fingers is not communicated to the blood drop.

Another method consists in introducing blood into a number of capillary tubes provided with rubber bulbs. At timed intervals after that, the bulbs are compressed (one tube at each interval) until it is found that clotting has occurred. When the unclotted blood is once moved in a tube by means of the bulb the further value of the tube in that test is impaired and it is to be laid aside.

Blood Plates.—These corpuscles as yet have little known clinical significance, and they are little investigated for practical purposes. Their examination requires special methods of preservation and staining.

Tests for Blood.—Occasion frequently arises for testing for the presence of blood in other organic fluids or dried stains. The following general tests are available:

Hæmin Test: This is applied to suspected dried stains, or to the dried residue or precipitate from suspected fluids. A drop of .6 per cent sodium-chloride solution is gently evaporated on a slide, a few finely divided particles of the suspected material are added to the salt, a cover-glass is laid over them, and a drop of glacial acetic acid is allowed to flow under the cover-glass. The specimen is gently heated for about a minute; as bubbles of gas form beneath the cover-glass more acetic acid is added. When the process is complete, or a faint brownish color appears, the acid is slowly evaporated and the specimen completely dried, holding it a little farther above the flame. The specimen is mounted in glycerin or glycerin jelly and examined with a high power of the microscope. In the presence of blood, hæmin crystals are formed by this process, appearing as minute brown rhomboid crystals.

Guaiacum Test: Four or five cubic centimetres of tincture of guaiacum is mixed with a third or half as much hydrogen peroxide (or with an equal amount of turpentine exposed for a long time to air and light,—"ozonized"). This mixture is then underlaid with the suspected fluid by the contact method; either the guaiacum mixture is allowed to flow gently down the side of the inclined test tube to overlie the fluid tested; or (better) the latter in a long glass tube or pipette, which is passed to the bottom of the guaiacum mixture, is allowed to flow out gently and underlie the other. The appearance, either immediately or after ten or fifteen minutes, of a robin's-egg-blue layer at the junction of the two fluids indicates the presence of hæmoglobin. Iodides or

iodine if present also give a blue color. If there is any difficulty in cleaning the glassware after this test the resinous precipitate may be very readily removed with spiritus ætheris nitrosi.

Spectroscopic Tests: The presence of hæmoglobin or derived pigments in suspected fluids may be very delicately and effectively revealed by spectroscopic methods, after proper treatment.

IV. THE STOMACH.

The examinations of the stomach which come within the province of the clinical laboratory are for the purpose of ascertaining the condition of the secretory, motor, and absorptive powers, and the occurrence of fermentative processes or pathological conditions of the stomach. The various distinct lines of examination are of the contents, the motor power, and the absorbent power of the stomach.

A. CHARACTERS OF THE STOMACH CONTENTS.

In the fasting condition (before meals) the stomach ordinarily contains only an insignificant amount of gastric juice, which is secreted in abundance only after the introduction of food.

The quantity of the contents of the digesting stomach varies according to the food and liquid ingested, the motor and secretory condition, and the capacity of the organ. The capacity of the stomach normally is very variable, ranging from 250 to 1,500 cubic centimetres; a capacity of over 1,500 cubic centimetres is usually due to dilatation, though a less volume may really exist in cases of naturally small stomachs dilated. The amount after Ewald's test meal ordinarily ranges up to about 75 cubic centimetres; more than this indicates motor insufficiency, delayed absorption, or hypersecretion.

Macroscopically the contents of the digesting stomach consist of the solid remains of the food, as yet undigested and undissolved, suspended in an abundant watery fluid. The latter consists of gastric juice with various soluble substances derived from the food or products of digestion in solution.

The filtered gastric fluid is ordinarily (at least after Ewald's meal) clear and almost colorless or with a faint greenish tinge. Bile gives it a green color, blood a red or brown color.

The odor is ordinarily faintly sour and aromatic. If volatile acids (as butyric) are present their characteristic odor is mani-

fested. Blood gives a peculiar smell, stercoraceous matter has a fæculent odor, necrotic conditions (as in carcinoma) give a foul odor. Special substances, as alcohol, laudanum, or phenol, may yield their characteristic odors.

Composition.—The chief constituents of the contents of the digesting stomach are:

Water (ingested and secreted).

Digestant substances secreted by the stomach: hydrochloric acid, pepsin, and rennet.

Ingested food materials and products of their digestion:

a. Crude food material as yet solid, undigested, and undissolved.

b. Soluble or digested food material in solution: proteids (albumin, syntonin, albumose, peptone), carbohydrates (starch, erythrodextrin, achroödextrin, maltose), acid salts, etc.

Products of fermentation generated by the action of micro-organisms on the food materials, especially of acid-forming bacteria on the carbohydrates; these are chiefly lactic, acetic, and butyric acids, and gases.

Drugs, foreign bodies, etc., ingested.

Adventitious and abnormal substances: saliva, microörganisms and other parasites, mucus, blood and blood corpuscles, pus, leucocytes, epithelium, fragments of gastric tissue, bile, and other material regurgitated from the small intestines.

Reaction and Acidity.—The gastric fluid is normally acid; pathologically it is sometimes neutral or alkaline. The acidity is due to the presence of hydrochloric acid, acid salts, and organic acids (lactic and fatty). Hydrochloric acid is ordinarily the chief acid principle; acid salts are usually present to a certain extent except when the fluid is neutral; organic acids may originate from fermentative processes.

In gastric work the degree of acidity is expressed quantitatively by a conventional scale, the unit or standard of which is the strength of a normal solution, which is put at 1,000. A fluid having an acidity of 50, for instance, would be one whose strength is $\frac{50}{1000}$ (or $\frac{1}{20}$) that of a normal acid solution; or one of which 100 cubic centimetres would be exactly neutralized by 50 cubic centimetres of a decinormal alkali solution.

Expressed on this scale, the total acidity of the gastric fluid obtained one hour after Ewald's test meal normally ranges from

about 40 to 65. A degree of acidity over about 65 is abnormal and excessive,—superacidity; acidity less than about 40 is abnormally low,—subacidity; complete absence of acidity, reaction neutral, is anacidity.

Hydrochloric Acid is normally present in the gastric fluid in the proportion of about .14 to .24 per cent (euchlorhydria). Abnormally it may be present in greater amount, in excess (hyperchlorhydria), in insufficient amount (hypochlorhydria), or absent altogether (achlorhydria).

The acid is present in two states, free and combined. The combined hydrochloric acid is that which has entered into combination with the albumin ingested to form acid albumin or syntonin, the free hydrochloric acid is that which is not in proteid combination.

Free hydrochloric acid does not appear in the gastric fluid until about thirty minutes after the ingestion of the Ewald meal, the acid first secreted immediately combining with the proteids. This acid tends to prevent bacterial activity, so that with an abundance of it in the stomach fermentation is usually not marked; but when it is deficient, fermentation may be active and organic acids generated that cause marked acidity of the gastric fluid. Immediately upon the ingestion of the Ewald meal lactic acid appears in abundance, derived partly from the food, partly from fermentation; but it progressively diminishes and disappears, while hydrochloric acid increases.

Pepsin is the proteolytic ferment secreted by the gastric glands, and is active only in acid media, especially dilute hydrochloric acid. It is secreted in the form of pepsin zymogen or pepsinogen, which becomes converted into active pepsin by the action of the hydrochloric acid.

Rennet is the other ferment secreted by the stomach, and curdles milk. It likewise is secreted in zymogen form, which is converted into active rennet by the action of hydrochloric acid or calcium chloride.

Achylia gastrica is the term applied to the condition in which the secretory power of the stomach is abolished and the essential elements of the gastric juice are not generated, hydrochloric acid, pepsin, and rennet being entirely absent or at most only a trace of rennet or its zymogen being present. This condition is especially present in atrophic or glandular gastritis and

gastric carcinoma, though it may sometimes be a neurotic manifestation.

Food Materials.—The soluble portions of the food ingested and the soluble products of digestion are dissolved in the gastric fluid. The insoluble and undigested portions occur as undissolved masses, macroscopic or microscopic in size, of vegetable tissue, starch granules, animal tissue (especially voluntary-muscle fibres), or fat.

Microscopically starch granules (Fig. 16) appear as oval bodies of variable size, some with faint concentric markings, some forms having an irregular cleft in the centre, of a rather dull lustre, and turning blue with iodine. Fragments of vegetable tissue (Fig. 18) present a characteristic appearance; groups of large cells with conspicuous cellulose cell-walls often forming regular geometrical patterns are frequent; also portions of sap ducts with spirally marked walls. Portions of muscle fibres are recognizable especially by their cross-striation.

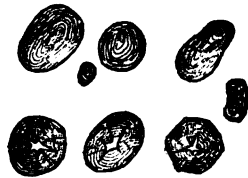


FIG. 16. — Starch Granules of Various Kinds.

Proteids may be present in all stages of digestion,—native albumins, acid albumin or syntonin, albumoses or propeptones, and peptone. In the absence of pepsin and hydrochloric acid, with consequent want of digestive power, albumose and peptone would not be formed, while after vigorous digestion the native proteids are entirely converted into peptone and propeptone.

Carbohydrates may also be present in all stages of digestion,—native starch, erythrodextrin, achroödextrin, maltose. Starch strikes a blue color with iodine, erythrodextrin a brown color, achroödextrin and maltose do not cause any change of color. While salivary digestion is checked by hydrochloric acid, after the Ewald test meal the dissolved starch is often entirely converted into erythrodextrin or sugar, the salivary digestion being completed before free hydrochloric acid appears in sufficient amount to check it. Dissolved starch is often absent from the gastric filtrate (having been entirely converted), while at the same time unchanged and undissolved starch may be present in large amount in the form of starch granules.

Fat ingested may be in the stomach unchanged, or the fatty

acids may be separated by the action of the hydrochloric acid of the gastric juice.

Acid salts (especially the acid phosphates of sodium and potassium) are ordinarily present, contributing materially to the acidity of the gastric fluid. They are derived from the food and partly perhaps as products of fermentation. The presence of excessive amounts of acid salts indicates excessive gastric fermentation.

Products of Fermentation.—Yeast fungi and bacteria are commonly present in the stomach, and have the power to cause fermentative decomposition of the carbohydrates (sugar), especially when hydrochloric acid is deficient or motor stagnation is present. The substances thus generated are especially lactic acid, certain fatty acids, and gases. Rarely alcohol may be generated from sugar by yeast fermentation in the stomach; acetone is at times found, and indol has been reported.

Lactic acid at times appears in the gastric fluid, partly introduced with the food, partly resulting from acid fermentation of sugars under the agency of micro-organisms. Lactic acid appears in the gastric fluid immediately after the ingestion of food, subsequently diminishing and disappearing as hydrochloric acid increases. Small amounts of lactic acid are introduced with the Ewald test meal; still, it is distinctly abnormal if lactic acid is demonstrable in the gastric fluid withdrawn one hour after this meal. If great nicety is desired as to lactic-acid formation, test meals are employed, such as that of Boas, containing no lactic acid. The presence of lactic acid in general indicates excessive gastric fermentation; while the combined occurrence of lactic and absence of hydrochloric acid are strongly indicative of gastric carcinoma.

Fatty Acids.—Certain fatty acids, especially butyric and acetic, may be present in the gastric fluid normally from the breaking down of fats introduced with the food, or abnormally from excessive fermentation of sugar in conditions like those generating lactic acid. Butter and fat having been excluded from the test meal, the presence of fatty acids is of pathological significance. These acids are odorous and volatile, and are dissipated by boiling.

Gases are quite constantly present in the stomach, originating from the swallowing of air, regurgitation from the small intes-

tine, or fermentation in the stomach. Excessive amounts or unusual kinds of gas indicate abnormal fermentation. The gases of the stomach are carbon dioxide, oxygen (in small amount, being absorbed by the blood with avidity), nitrogen, hydrogen, and exceptionally methane, ammonia, hydrogen sulphide (the last three in proteid fermentations).

Drugs, etc., ingested may be demonstrable in the gastric contents, sometimes aiding in diagnosis in cases of poisoning. Thus laudanum, phenol, alcohol, and other substances may be detected by their odor or other tests in vomitus or stomach washings.

Foreign objects like coins are frequently swallowed. Calculous masses, chiefly formed by an aggregation of insoluble ingested material, are rarely found in the stomach.

Parasites and Micro-organisms.—Occasionally various intestinal parasites find their way into the stomach, or animal organisms may be swallowed; in a few cases the presence of insect larvæ has caused gastritis. Vegetable micro-organisms are ordinarily present in the stomach, where they may cause fermentative processes. Owing to its acidity the gastric fluid is an unfavorable medium for the growth of micro-organisms, and they are far less abundant in this organ than in the intestines; they flourish more abundantly and fermentation processes are most active in the stomach when there is a deficiency or absence of hydrochloric acid or motor stagnation.

The vegetable parasites of the stomach belong to the fungi and bacteria. Fungous forms not infrequently occur in the stomach contents, rarely with any pathological significance; gastric lesions due to the growth of fungi are exceedingly rare, though gastritis due to *favus* and *oidium albicans* has been reported. *Saccharomycetes*, or fungi of the yeast group, are common and normal inhabitants of the stomach, at least after bread diet; they appear as oval bodies, 3 to 10 micromillimetres long, often growing or budding from one another in chains of three or four each (Fig. 17).

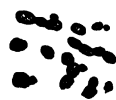


FIG. 17.—Yeast Fungi.

Important among the bacteria are those capable of causing lactic and other acid fermentations, *sarcinæ* (in the form of cubical packets), and the *Oppler-Boas bacillus*.

The *Oppler-Boas bacillus* is a large, long, non-motile bacillus, similar in size and form to the hay bacillus; it sometimes grows

in long filaments, and at other times the bacilli are crowded together in large dense masses. The presence of this bacillus is strongly indicative of gastric carcinoma.

Saliva is commonly present in the stomach, at times in excessive amounts.

Mucus.—Mucin is normally secreted by the gastric mucosa in amount sufficient to give the gastric fluid a somewhat mucinous consistency. In catarrhal conditions of the stomach mucus may be produced in excessive amount.

Snail-shell bodies, small spiral objects of microscopic size, single or in clusters, are sometimes seen, especially in the fluid from the fasting stomach; they are supposed to be formed by the action of hydrochloric acid on mucin, and have no special significance.

Blood appears in the gastric contents in hæmorrhagic conditions, especially ulcer and cancer. It may vary in amount from a small quantity only sufficient to give the gastric fluid a reddish or brownish tinge, to very large amounts. It may be bright red, fresh and unaltered in appearance, or if it remains a long time in the stomach it may undergo change to a dark-brown color. The red corpuscles, if not broken down, will appear microscopically.

Pus rarely appears in the stomach contents, from phlegmonous gastritis or rupture of abscesses into this viscus.

Leucocytes, which are apt to be altered by the action of the gastric juice, may be present in small numbers without indicating anything abnormal. In large numbers they indicate catarrhal or suppurative processes.

Epithelium cells are frequently present in the gastric contents. Squamous cells from the œsophagus or pharynx are commoner than columnar cells derived from the stomach itself. Cells from carcinomatous growths may also be present, but when separated and isolated from one another it is impossible to recognize them with certainty as originating from abnormal growths. All these cells are apt to be altered by the action of the gastric juice, being frequently swollen and clear; or the nuclei may be free.

Fragments of gastric tissue torn off by the stomach tube or loosened by ulceration are at times found in the gastric contents, being portions either of the gastric mucosa or of neoplasms. The determination of their exact nature may be of great aid in

diagnosis as to the form of gastritis or tumor present. Fragments of the stomach mucous membrane may signify ulceration, though they do not always indicate anything very serious.

Materials regurgitated from the small intestine are frequently present in the stomach, especially bile. Leucin, tyrosin, cholesterolin, pancreatic juice, etc., may be present in association with bile, being recognizable by chemical tests, or microscopically by crystalline forms. In intestinal obstruction abundant stercoraceous or fæcal material may be regurgitated into the stomach and vomited.

Bile in the stomach is usually manifested by a bright-green color, due to biliverdin formed from bilirubin by the action of the hydrochloric acid.

B. EXAMINATION OF THE STOMACH CONTENTS.

The examination of the stomach contents may be conveniently divided into four classes of work: (1) examination of the stomach contents during digestion or after test meals; (2) examination of the contents of the stomach while fasting; (3) examination of stomach washings; and (4) examination of vomitus.

For convenience in systematizing and recording the results of stomach-contents examinations some such blank form as that on the following page can be used.

1. Examination of Stomach Contents during Digestion.

One of the chief objects of clinical gastrology is to determine the secreting power of the stomach, through chemical analysis of the gastric juice. As this fluid is ordinarily obtainable in sufficient quantity only after the ingestion of food, in order to obtain a sample it is necessary to take it after a meal; the presence of food materials and products of digestion does not for clinical purposes interfere with the analysis of the gastric juice, but even affords additional information as to fermentative processes, etc. To obtain uniform and comparable results standard or test meals are given, and the stomach contents removed with a stomach tube after a definite interval. Examination of the stomach contents after test meals comprises by far the largest part of laboratory gastrological investigation; the chief drawback is the difficulty and discomfort of obtaining the specimens.

Date, ... 190 .

EXAMINATION OF STOMACH CONTENTS.

Name of patient.....

Nature of specimen.....
 [Stomach contents after test meal, in fasting condition, stomach washings, or vomitus.]

General characters.....

Quantity.....c.c. Reaction.....

Total acidity.....

Acidity due to free hydrochloric acid.....

Acidity due to combined hydrochloric acid.....

Acidity due to organic acids.....

Acidity due to acid salts.....

Hydrochloric acid [present or absent]; percentage.....
 free hydrochloric acid.... per cent; combined hydrochloric acid.... per cent.

Lactic acid..... Fatty acids..... Acid salts.....

Pepsin..... Pepsinogen.....

Rennet..... Rennet zymogen.....

Native albumin... Acid albumin... Propeptone.... Peptone.....

Starch..... Erythrodextrin... Achroödextrin.. Sugar.....

Blood..... Bils..... Mucus.....

.....

.....

Microscopical examination:

Food material.....

Starch granules..... Fat.....

Micro-organisms.....

Amorphous material..... Snail-shell bodies.....

Red blood corpuscles..... Leucocytes.....

Epithelium.....

Tissue fragments.....

.....

.....

Remarks.....

.....

.....

.....

.....

(Signature)

The chief items in the technique of examination of the stomach contents after test meals are as follows:

Test meals.

Obtaining the stomach contents.

Macroscopic examination.

Qualitative tests for hydrochloric, lactic, and fatty acids.

Quantitative determination of amount of total acidity, free and combined hydrochloric acid, organic acids, and acid salts.

Tests for pepsin and pepsinogen.

Tests for rennet and rennet zymogen.

Tests for proteids.

Tests for carbohydrates.

Tests for blood, bile, mucus.

Microscopical examination.

Bacteriological examination.

Test Meals.—*Ewald's test meal* is the one in most general use, consisting of 35 to 70 grams of wheat bread (a roll or one or two slices of bread), without butter, and 300 to 400 cubic centimetres of water or weak tea without sugar. This is best given in the morning, on an empty stomach, and nothing else is to be ingested, before or after, till the stomach is emptied. If there is motor stagnation of the stomach, it may be advisable to wash out the organ the evening previous. The contents are to be removed one hour after the ingestion of the meal, the digestive activity being at this period at its maximum.

Boas's test breakfast is employed when it is required to determine very carefully whether lactic acid is generated in the stomach. This meal contains no lactic acid, while Ewald's meal contains small amounts. Boas's meal consists of a tablespoonful of rolled oats added to a litre of water, with a little salt if desired, and the whole boiled down to half a litre. The stomach is washed out the night before, the meal is given in the morning, and the gastric contents are withdrawn an hour later.

Obtaining the Stomach Contents.—This is done by means of the stomach tube, avoiding admixture of saliva or water. The tube is of soft rubber, about a centimetre in diameter and one and a half metres long. The gastric end should have an opening at the tip and one or two lateral openings. To pass the tube, which is lubricated with oil or simply moistened outside, dry inside, its point is passed in against the pharyngeal wall and the buccal

firmly and steadily pushed onward until it enters the stomach. A mark on the tube, about 50 centimetres from the end, is brought about even with the teeth, when the end of the tube will ordinarily be within the stomach. The passage of the tube will be facilitated if the patient aids by swallowing motions, or breathes regularly and deeply. The operation at the first attempt is usually attended with difficulty and discomfort, and a copious flow of mucus and saliva is excited; but with firmness it is usually successfully accomplished.

The tube passed, the next step is to empty the stomach. The outer end of the tube should be held at a lower level than the stomach, so that when the flow is once started it will continue by siphon action. The flow is started by having the patient contract or strain with his abdominal muscles or by making the motions of coughing. If this fails, moving the tube rapidly to and fro a few times up and down the œsophagus may start it. At times it may be necessary to employ aspiration, a rubber bulb being interposed in or attached to the tube, or the tube being connected with an aspirating bottle from which the air is exhausted by a Politzer bag, the lips, or otherwise; or the tube may be "stripped" by the hands in a manner analogous to that employed in milking cows. If failure to flow is caused by particles of food blocking the tube, by blowing in the tube these may be dislodged and the passage cleared. The stomach contents are received in a clean, dry receptacle, saliva flowing down the outside of the tube being prevented from mixing with the gastric fluid. The tube should be tightly pinched while being withdrawn to prevent fluid within it from passing into the larynx.

The use of the tube is contra-indicated in cases of thoracic aneurism, severe cardiac disease, recent gastric hemorrhages, etc.

After being obtained the specimen is filtered through dry filter-paper. This is often a rather slow process, but may be hastened by the use of a filter-pump.

Macroscopic Examination.—The specimen thus obtained consists of particles of bread suspended in a liquid. In achylia gastrica the bread is often less finely divided than in normal conditions. The liquid portion or filtrate is clear, normally almost colorless. The presence of masses of mucus, blood, bile, tissue fragments, etc., may be evident to the unassisted eye. The odor may give some evidence as to the presence of organic

acids, blood, necrotic matter, etc. The total quantity of gastric contents obtained ordinarily ranges up to 50 or 75 cubic centimetres.

Qualitative Test for Free Acids.—The presence of free acids (hydrochloric or organic) or of combined hydrochloric acid may be determined by adding a few drops of a one-half-per-cent aqueous solution of Congo red. This in the presence of free acids or combined hydrochloric acid turns dark-blue or blackish-brown, is unchanged by acid salts, and in neutral or alkaline solutions is red. In practice this test will not often be needed, except that in subacid gastric fluid a negative result may be significant of absence of free or combined hydrochloric acid and of organic acids.

Qualitative Tests for Hydrochloric Acid.—1. *Töpfer's Test*: Add to a few drops of the gastric filtrate a drop or two of a .5 per cent solution of dimethylamidoazobenzol in alcohol; in the presence of hydrochloric acid a bright-red color results, in its absence a bright-yellow. Minute traces of hydrochloric acid yield an intermediate brownish color. This test is very delicate and simple and can be made in connection with the quantitative analysis; in the stomach contents no other acids than hydrochloric are present in sufficient amounts to give the reaction. The test is sensitive to hydrochloric acid diluted to about .01 per cent.

The two following tests were formerly much used, but are less convenient than Töpfer's test.

2. *Gunzberg's Test*.—To two or three drops of the gastric filtrate in a porcelain evaporating dish add a like amount of a solution of phloroglucin 2 grams, vanillin 1 gram, in absolute alcohol 30 c.c. Gently evaporate the mixture over a Bunsen burner. If hydrochloric acid is present a rose-red color appears at the margin of the fluid as it evaporates; if absent a yellow or brown color only is left. The test solution is expensive, rather unstable, and should be kept in the dark.

3. *Boas's Test*.—The test solution consists of resorcin resublimed 5 grams, white sugar 3 grams, 95 per cent alcohol 100 c.c. The method of applying the test and the results are precisely the same as by Gunzberg's method.

Qualitative Tests for Organic Acids.—Lactic acid and the fatty acids are soluble in ether, and may be separated from gastric fluid by shaking the latter with five or ten times its volume of pure neutral ether. Tests may be applied to the material obtained in such ethereal extracts to determine the presence, kind,

and amounts of organic acids, but the methods are rather troublesome and not often used. If the ethereal extract thus prepared reacts acid with litmus paper, the presence of organic acids is shown. If weakly acid fluid gives no reaction with Congo red, organic acids are absent.

Qualitative Test for Lactic Acid.—The ordinary tests for lactic acid are not as delicate or as distinct in their results as is desirable. If the presence of organic acid is shown by the ether method, lactic acid may be assumed to be present. If a very careful determination as to the presence of lactic acid is desired Boas's test meal is to be employed rather than Ewald's, though normally lactic acid is not demonstrable by Uffelmann's method in the fluid withdrawn an hour after Ewald's meal.

Uffelmann's test is the one commonly employed. The test solution is prepared freshly as needed by adding a drop of a weak watery solution of ferric chloride (FeCl_3) to a few cubic centimetres of a 2-per-cent phenol solution, diluting till the mixture is of a light-blue or amethyst color. To two or three cubic centimetres of this fluid a like amount of gastric filtrate is added, when if lactic acid is present the liquid turns a lemon or canary-yellow color; otherwise the color is unchanged or turns to a grayish. With pure solutions of lactic acid this test is sensitive to about .01 per cent, but in the presence of hydrochloric acid and other substances in solution in the gastric fluid its sensitiveness and certainty are much impaired. To overcome this source of difficulty an ether extract of the gastric fluid may be made, and the material thus separated may be tested by Uffelmann's method.

Strauss's Method: Gastric filtrate is poured into the special apparatus used for this test up to the mark indicating 5 cubic centimetres, and ether is then added to the 25 cubic-centimetre line. After shaking thoroughly, the fluids are allowed to separate, and the stopcock is opened and the fluid allowed to escape till the 5 c.c. line is reached. Water is added up to the 25 c.c. mark; two drops (measured by a dropper for uniformity) of a mixture of 1 part of the official tincture of ferric chloride diluted with 9 parts of water is added, and the whole agitated. In the presence of lactic acid the mixture turns green, bright green if .1 per cent or more of lactic acid was present, pale green if between .05 and .1 per cent. This affords a rough approximate

judgment of the amount of lactic acid. It is negative with traces of lactic acid too minute to be of clinical significance.

Qualitative Tests for Fatty Acids.—The odor often gives sufficient indication of the presence of fatty acids (butyric, acetic), especially when heated. As these acids are volatile and dissipated by heat, their presence is indicated if moistened blue litmus paper turns red in the vapor rising from the gastric filtrate while being boiled; or if there is a loss of acidity after boiling the filtrate.

Quantitative Determination of the Acids.—The most important points to be determined are the amount of the total acidity and of free hydrochloric acid; the amount of combined hydrochloric acid, the total amount of free acids, of the organic acids, of lactic acid, of fatty acids, and of the acid salts may also be determined. The estimation of the amount of the various acid factors is accomplished by titrating with decinormal sodium-hydrate solution, using different substances as indicators for the various purposes.

Normal and Decinormal Solutions.—A normal solution of any reagent, as the term is used in volumetric analysis, is one containing in a litre enough of the reagent to yield, combine with, replace, or correspond to one gram of hydrogen. For monobasic acids, monacid bases, or their salts, a normal solution is one in which the number of grams in the litre is equal to the molecular weight of the reagent, or in other words one whose percentage strength by weight is equal to one-tenth of the molecular weight; thus, a normal solution of hydrochloric acid (molecular weight 36.37) is one containing 36.37 grams of the acid in the litre, or of 3.637 per cent strength; one of sodium hydrate 3.996 per cent, one of sodium chloride 5.837 per cent, one of lactic acid 8.979 per cent, etc. For normal solutions of dibasic or tribasic acids and compounds, etc., the number of grams to the litre is equal to the molecular weight divided by 2, 3, or in general by the number of atoms of active and replaceable hydrogen (or hydroxyl radicles) in the molecule; thus, a normal solution of oxalic acid (dibasic, molecular weight 89.78) is of 4.489 per cent strength, one of phosphoric acid (H_3PO_4 , tribasic, molecular weight 97.80) 3.26 per cent, etc.

From their composition it follows that equal volumes of normal (or decinormal, etc.) solutions are exactly equal in their acid or alkaline strength; for instance, equal amounts of any normal acid and basic solutions exactly neutralize each other.

Decinormal solutions are those of one-tenth the strength of the corresponding normal solutions; centinormal and millinormal solutions are of one-hundredth and one-thousandth the strength of normal solutions respectively.

This mode of expression can be extended so as to afford a very satisfactory general method of expressing the degree of acidity or alkalinity of any organic

or other fluid. The strength of a normal solution being taken as the standard or unit, and designated as 1 (or 1,000), other solutions are designated by numbers (decimals or integers) indicating their fractional strength relative to the normal solution. The standard might be designated by the letter N. Thus, an acidity expressed as .001, or .001 N, or $\frac{N}{1,000}$ (or 1 if the standard be expressed as 1,000), would mean that the acid strength of the fluid is one-thousandth that of a normal acid solution; an alkalinity of .022 (or .022 N, or 22 on a scale of 1,000) would represent the alkalinity of a solution twenty-two-thousandths the strength of that of a normal alkaline solution; a fluid of which 10 c.c. would be neutralized by 4.5 c.c. of decinormal alkali would have an acidity of .045 N (or 45 on the 1,000 scale).

The scale of notation in which the normal solution is taken as 1 would probably be for general use the most satisfactory and least misleading mode of expression, but in stomach work the scale in which the normal is fixed at 1,000 has come into general use. Thus in gastrology an acidity of 5, 45, or n , means an acid strength of 5, 45, or n thousandths, respectively, of that of a normal acid solution; or that 100 cubic centimetres of the gastric fluid would be exactly neutralized by 5, 45, or n cubic centimetres of decinormal sodium-hydrate solution.

The amount of acidity or alkalinity produced by a substance in solution being known, the percentage strength, by weight, of the substance can be readily calculated from the amount required to make a normal solution. Thus, since a normal solution of hydrochloric acid consists of 3.637 per cent of the absolute acid, a solution of hydrochloric acid of a strength equal to $\frac{m}{n}$ that of normal would have a strength of $\frac{3.637}{n} m$ per cent by weight; or each one-thousandth of acidity would represent .003637 per cent of hydrochloric acid. Solutions of lactic acid of strength of $\frac{m}{n}$ that of normal would contain $\frac{8.979}{n} m$ per cent of lactic acid by weight; or each one-thousandth of acidity would represent .008979 per cent of the acid. These factors are used in gastric work to calculate the weight percentages of these acids when the degree of acidity due to them is determined by titration.

In preparing any volumetric test-solution the weight of the substance used must be very exact; and it is necessary to take into consideration the presence or absence of water of crystallization or of deliquescence or efflorescence of water in weighing out the substance to be dissolved. Chemically pure, dry, well-formed crystals with a known content of water of crystallization must be taken when the solution is prepared by direct weighing; thus, a decinormal solution of oxalic acid may be made by dissolving 4.489 grams of the perfect, dry, and pure crystals of this substance in sufficient water to make a litre. In the case of liquid substances like hydrochloric acid normal solutions may be prepared by very precise gravimetric or specific-gravity methods. In the case of substances that effloresce or deliquesce, thus making the content of water an uncertain quantity, an approximate solution is prepared first and then brought to the required strength after comparison or titration with a standard volumetric solution.

Preparation of Decinormal Sodium-hydrate Solution.—This is the solution employed in quantitative estimation of the gastric acids. Sodium hydrate so rapidly absorbs water from the atmosphere that exact amounts of it cannot be measured out by direct weighing. Decinormal solutions are therefore prepared by indirect methods. To prepare a decinormal solution about 4 grams of sodium hydrate are dissolved in eight or nine hundred cubic centimetres of water, thus making a solution rather stronger than decinormal. The exact alkalinity or strength of this solution is then determined by titration with normal or decinormal hydrochloric acid (which may be purchased ready prepared) or with decinormal oxalic-acid solution (which may be prepared in the laboratory). The strength of the preliminary solution being ascertained, the amount of water necessary to make the solution of decinormal strength is then calculated and added. The solution is again tested, and if necessary further changes are made until it is exactly decinormal in strength. From time to time it is well to compare the stock decinormal sodium-hydrate solution with the standard acid solution to see that it does not deteriorate.

Technique of Titration.—The process of acidimetric and alkali-metric titration consists essentially in adding to a known amount of the fluid to be tested measured quantities of a standard volumetric solution of the opposite reaction until exact neutralization is attained, which is shown by visible color changes manifested by "indicators" also added. The test fluid (decinormal soda in the case of stomach work) is contained in a burette, or glass tube of about 50 cubic centimetres capacity graduated to tenths of a cubic centimetre, clamped upright to a support. The lower end of the burette is contracted to a point through which the test fluid can be discharged drop by drop, the flow being controlled or checked altogether by means of a perforated glass stopcock, or by a pinchcock on a rubber tube connecting the end of the burette with a glass tube drawn to a point. Beneath the burette is placed a beaker—or, better, a porcelain dish or evaporating dish, the white color of which affords a better background for observing the color changes. Into this dish is put a definite amount of the gastric fluid or other fluid to be tested, along with a few drops of the solution of the "indicator" employed. Various indicators are employed for different purposes. Phenolphthalein, for instance, is colorless in presence of acids, bright

rose-red in alkaline media; in titrating gastric fluid, the decinormal sodium hydrate is to be added until a permanent pink is attained, which indicates neutralization; in titrating alkaline fluids, decinormal acid is added until the red color of the phenolphthalein just disappears.

The degree of acidity or alkalinity of the fluid tested is determined from the amount of this fluid employed in the test, the amount of the test fluid required for neutralization, and the known volumetric strength of the latter. The number of cubic centimetres of the one fluid, multiplied by its acidity, is equal to the number of cubic centimetres of the other fluid, multiplied by its alkalinity. The number expressing the degree of acidity or alkalinity required may therefore be found by this rule: Multiply the amount of the test solution used in the titration by its alkalinity or acidity (expressed in terms of its fractional normal strength), and divide the product by the amount of the fluid tested that was employed; the quotient is the degree of acidity or alkalinity of the latter.

In actual gastric work the burette should be first rinsed out with a small amount of the decinormal sodium-hydrate solution to be used, and then enough of this solution should be poured into the burette so that the upper level of the fluid stands somewhere in the graduated portion of the tube; the stopcock or pinchcock at the lower end of the burette should be opened for a few seconds to allow the portion of the tube beneath the cock to become completely filled with the test solution; before titrating, a few minutes should be allowed to elapse to permit the fluid adhering to the sides of the upper empty part of the burette completely to settle into the column of liquid. A definite amount of gastric filtrate (2, 5, or 10 cubic centimetres according to the amount available) is then accurately measured out by means of a volumetric pipette and placed in a clean white porcelain dish or beaker beneath the burette, and three or four drops of the indicator solution is added. The upper level of the fluid in the burette is then noted, reading the graduation even with the lowest limit of the concave surface of the test fluid. The test fluid is then allowed to flow drop by drop into the gastric fluid below, the discharge being controlled by the cock. The fluid beneath is at the same time stirred with a clean glass rod. When the end reaction, as shown by the requisite color change of the in-

indicator, is attained the flow is stopped, and the level of the test fluid in the burette is again noted. The difference between the two readings of the height of the column of test fluid gives the number of cubic centimetres of this fluid required to neutralize the gastric acid; from this quantity and the quantity of gastric filtrate in the beaker, the degree of acidity of the latter can be calculated.

As already stated, it is customary to state the degree of acidity of gastric fluid by the fractional normal strength, a normal acid solution being 1,000; or, as it is usually expressed, by the number of cubic centimetres of decinormal sodium-hydrate solution that would be required to neutralize 100 cubic centimetres of the gastric acid. To find this number, multiply the number of cubic centimetres of the decinormal soda solution used by 100, and divide the product by the number of cubic centimetres of the gastric fluid employed.

Determination of the Total Acidity of Gastric Fluid.—This is done by titration with decinormal soda in the manner described, using as an indicator a 1-per-cent solution of phenolphthalein in 50-per-cent alcohol. This substance is reddened by alkalis, colorless in all acid media; the titration is continued till a permanent pale pink is reached. The result expresses the total acidity of the gastric fluid, due to all the acid factors together, free acids, hydrochloric acid in proteid combination, and acid salts alike. The total acidity after the Ewald meal is normally about 40 to 65.

Determination of Free Hydrochloric Acid.—A few drops of a .5-per-cent alcoholic solution of dimethylamidoazobenzol is added to the measured amount of gastric filtrate. If a pure yellow color results, like that produced by adding a little of the test solution to pure water, hydrochloric acid is absent, and no titration is necessary. If a cherry-red or brownish color results, hydrochloric acid is present, and the degree of acidity due to it may then be determined by titrating with the soda solution till the red or brownish color is replaced by a pure yellow, which indicates the complete neutralization of the hydrochloric acid (this acid combining with the sodium hydrate before the other acids do). Normally the acidity due to free hydrochloric acid is about 40 to 60.

By Mintz's method, the titration is conducted until a small drop removed

from the liquid fails to give the reaction for hydrochloric acid by Gunzberg's or Boas's test.

To calculate the actual weight percentage of free hydrochloric acid from its acidity, multiply each unit (or thousandth) of acidity due to it by .003637; the product expresses the percentage by weight of free hydrochloric acid. Thus, if the acidity due to hydrochloric acid is 45, the proportion of the acid present is $45 \times .003637$, or .163665 per cent.

After neutralizing the hydrochloric acid by titration in the manner described, phenolphthalein may be added and the titration continued to make a second and corroborative determination of the total acidity. The addition of a second indicator after the first has accomplished its purpose is feasible in certain cases, and economizes time, labor, and gastric fluid.

Determination of Combined Hydrochloric Acid.—*Alizarin method:* The indicator used for this purpose is alizarin sodium sulfonate in 1-per-cent aqueous solution. Mixed with neutral or acid liquids, this strikes a yellow color; with sodium hydrate it gives a pure violet color. The solution should be tested with sodium-hydrate or sodium-carbonate solution in order to learn the proper color of the end reaction. Alizarin reacts with free acids and acid salts, but not with combined hydrochloric acid. To determine the latter, the gastric fluid is titrated with the alizarin solution as indicator; the difference between the acidity so obtained and the total acidity obtained with phenolphthalein is the amount of acidity due to hydrochloric acid in proteid combination. The weight percentage of the acid can then be calculated as in the case of the free acid. The method is not very satisfactory, as the end reaction is rather vague.

Leo's method, which indicates alike the amount of acid salts and that of free acids plus combined hydrochloric acid, depends on the fact that calcium carbonate combines with and neutralizes free acids and combined hydrochloric acid, but does not affect the acid salts. The formation and presence of calcium chloride, however, interfere with the action of phenolphthalein, so that in titrating it is necessary to add an excess of this salt. A portion of the gastric fluid is first mixed with half as much neutral concentrated solution of calcium chloride, and titrated with phenolphthalein. With another portion of the gastric fluid a small amount of dry, powdered, pure neutral calcium carbonate

is mixed and stirred; the mixture is filtered through a dry filter; a measured portion of the filtrate is taken, air is forced through it to remove the carbon dioxide generated, half as much concentrated calcium-chloride solution is added, and it is then titrated with phenolphthalein. The difference between the two results gives the acidity due to free acids and combined hydrochloric acid; and deducting this acidity from the total acidity of the gastric fluid, as determined in the ordinary manner, gives the acidity due to acid salts. From the combined acidity due to free acids and combined hydrochloric acid, deducting the acidity due to free acids as obtained by other methods gives the acidity of the combined hydrochloric acid.

Other methods of determining chlorine in its acid and neutral combinations are available, but rather too complicated for general use.

Determination of Organic Acids.—The organic acids may be removed from the gastric fluid by extraction with ether, and the amount of acidity so lost represents the acidity due to organic acids. A portion of the gastric filtrate is briskly agitated with a quantity of neutral ether; the fluids are allowed to separate, and the gastric fluid is shaken with another portion of ether. This process is repeated until the gastric fluid has been treated with five or ten times its quantity of ether. A measured portion of the fluid is then titrated with phenolphthalein and the decrease of acidity represents the organic acids. The ethereal extract can also be tested for organic acids, the amount found present corresponding to the amount of gastric fluid originally used.

Determination of the Fatty Acids.—As the fatty acids are volatile, a measured amount of gastric fluid is boiled for a long time, the water lost being replaced as necessary; it is then diluted to the original volume, and titrated with phenolphthalein. The loss of acidity, if any, after boiling, as compared with the total acidity of the unboiled fluid, represents the acidity due to the fatty acids. As, it is said, some hydrochloric acid is lost by boiling, it may be necessary to determine the amount of this acid before and after boiling, and take this factor into account.

Determination of Lactic Acid.—The difference between the acidity due to the total organic acids and that due to fatty acids represents the acidity due to lactic acid. In the absence of fatty

acids the organic acids are represented by lactic acid alone. The percentage amount of lactic acid can be calculated by multiplying the number expressing its acidity by .008979.

Other methods of determining lactic acid are available, but are rather complicated and do not appear to be much used.

Determination of Acid Salts.—The acidity due to acid salts is the difference between the total acidity and the acidity due to free acids and combined hydrochloric acid, and may be so determined.

Tests for Pepsin and Pepsinogen.—This test requires the use of small pieces of fibrin or white of egg well coagulated by boiling, cut in sizes of 4 or 5 millimetres; a quantity of these may be kept on hand in a bottle of glycerin, sterilized in the steam sterilizer for half an hour, ready for instant use. If the gastric fluid to be tested for pepsin contains hydrochloric acid, a piece of the coagulated albumin is removed from the glycerin, rinsed with water, placed in a test tube with 5 or 10 cubic centimetres of gastric filtrate, and set aside in an oven at 37° C. If the pepsin is normal, the bit of coagulated albumin should be entirely digested and dissolved in six or seven hours. If pepsin is deficient, the albumin will require a longer time to be digested; while if it is absent altogether the albumin will not be digested at all after a prolonged period.

If hydrochloric acid is absent from the gastric fluid, .1 or .2 per cent of the acid should be added to convert pepsinogen into pepsin and present the proper conditions for digestion. Its digestive power is then tested with a piece of coagulated albumin in the manner just indicated.

Tests for Rennet and its Zymogen.—To test for rennet, a few drops of gastric fluid are mixed with about 15 cubic centimetres of milk in a test tube, and set away at a temperature of about 37° C. If rennet is normal, the milk will be coagulated solid, or with the separation of a small amount of clear whey, in ten or fifteen minutes. If the milk curdles more slowly, rennet is deficient.

If hydrochloric acid is absent from the gastric fluid, add a small amount of calcium-chloride solution to the mixture of milk and gastric juice used in the rennet test. Rennet zymogen if present is then converted into active rennet, which coagulates the milk.

Tests for Proteids.—The ordinary tests for proteids are used in stomach work.

Acid albumin or syntonin if present will be precipitated on carefully neutralizing the gastric filtrate; the precipitate redissolves with an excess of either alkali or acid.

Albumin.—After removing syntonin by neutralizing and filtering, boil the filtrate or apply the nitric-acid contact test; a cloudiness indicates unchanged albumin. Boiling the unneutralized gastric filtrate precipitates albumin and syntonin together, which may then be removed by filtering.

Albumose or propeptone.—After removing albumin and syntonin by boiling and filtering, mix equal quantities of the cooled filtrate and a saturated solution of sodium chloride, and add a drop or two of acetic acid; a turbidity or precipitate in the cold, disappearing on heating and reappearing when again cooled, indicates the presence of albumose. The filtrate should also give the biuret reaction. The test may also be tried with unboiled gastric filtrate; a turbidity diminishing with heat indicates albumose.

Peptone.—Albumin, syntonin, and albumose being removed, the remaining filtrate, which should give a negative result when tested by acetic acid and potassium ferrocyanide, is subjected to the biuret test; a violet-red or purplish color indicates the presence of peptone. Tannin and other substances should also give a positive result.

Tests for Carbohydrates.—To a few cubic centimetres of Lugol's or Gram's iodine-iodide solution (page 11), diluted to a light yellow color, add a few drops of gastric filtrate; the formation of a blue color indicates the presence of unchanged *starch*, the darkening of the color of the iodine solution to a deep brown or mahogany indicates the presence of *erythrodextrin*. The presence of *sugar* may be tested for by the ordinary glucose tests, as the copper test. With a faint reaction for sugar and in the absence of starch and erythrodextrin, *achroödextrin* may be assumed to be present.

Tests for Blood.—The presence of blood may be obvious from the appearance of the specimen, a reddish or brownish color of the filtrate, or the presence of red corpuscles. If desirable the guaiacum or hæmin test can be applied.

Tests for Bile.—The presence of bile is ordinarily indicated by

a green color of the fluid, due to the formation of biliverdin from bilirubin by the action of hydrochloric acid. The nitric-acid contact test, by the formation of a green zone, indicates the presence of bile.

Mucus if in excess is sufficiently obvious to macroscopic examination.

Microscopical Examination.—A representative sample of the solid sediment of the stomach contents is taken with a glass tube, placed on a slide, covered with a cover-glass, and examined with both low and medium powers. This may reveal the presence of undissolved food material, starch granules, fat globules, fatty-acid crystals, micro-organisms (fungi, saccharomycetes, bacteria), red blood corpuscles, leucocytes, tissue fragments, snail-shell bodies, and leucin, tyrosin, or cholesterin regurgitated from the intestine. Ordinarily, after the Ewald test meal the microscopical field is mostly made up of a crowded mass of starch granules, with a few yeast fungi and bacteria, and perhaps some squamous epithelium cells. By allowing a drop of iodine solution to run under the cover-glass, the starch granules are colored deep blue and thus differentiated from other objects. By similarly adding a drop of Sudan III. solution, fat globules are colored orange or red and thus differentiated. Bacteria and cells may be stained in the ordinary way if desired. Fragments of tissue may be hardened and sectioned in the usual way to determine their histological structure.

Bacteriological Examination.—If desired, cultures may be made from the stomach contents (removed with aseptic precautions) to determine fungi, saccharomycetes, and bacteria.

2. Examination of Stomach Contents during Fasting.

To determine if there is an excessive secretion of gastric juice during the fasting as well as the digesting period (*succorrhœa gastrica*), the stomach should be washed out and emptied in the evening, and its contents removed in the morning, no solids or liquids being ingested in the mean time. The fluid so obtained should be measured, and its content in hydrochloric acid, pepsin, mucus, etc., determined by the methods given above. Normally the fasting stomach contains only a few or at most 50 or 60 cubic centimetres of fluid; an amount in excess of this indicates hypersecretion.

3. Examination of Stomach Washings.

At times the stomach contents are removed by the tube under other circumstances than after the test meal, and chemical or microscopical examination on the lines above given may be desirable. Thus, in emptying the stomach after the ingestion of poison, examination of the fluid removed may reveal whether and what poison has been taken.

Occasionally in the fluid used in washing out the stomach particles of solid matter are obtained, the microscopical examination of which may be of diagnostic usefulness.

4. Examination of Vomitus.

Vomited material is ordinarily so mixed with saliva or pathological products that its examination is unsatisfactory so far as the secretory powers of the stomach are concerned, though pepsin and rennet may be demonstrable. The detection of remnants of food material, dissolved proteids or carbohydrates, ingested drugs, products of fermentation (organic acids, etc.), micro-organisms, mucus, blood or transformed hæmoglobin, leucocytes, epithelium, fragments of gastric tissue, bile, stercoraceous material, etc., by macroscopic, chemical, or microscopic examination may afford valuable diagnostic information. An excessive amount of hydrochloric acid may indicate hyperchlorhydria. The reaction may be alkaline from the presence of blood or bile. The food materials present may be significant as to the motor power of the stomach, and to a certain extent as to its digestive power.

In suspected stricture of the œsophagus it may be important to determine whether regurgitated ingested material comes from the stomach or the œsophagus. The presence of rennet, pepsin, free hydrochloric acid, or bile pigment would signify that the material came from the stomach. Their absence would indicate either œsophageal obstruction or achylia gastrica. The reaction is no sure criterion.

Vomiting of swallowed saliva sometimes occurs.

C. EXAMINATION OF MOTOR POWER OF STOMACH.

Normally an ordinary meal should be digested and passed on into the intestine in six or seven hours. Motor insufficiency causing retention of food material in the stomach an abnormally

long time, up to several days, is an important factor in some gastric diseases, as pyloric stricture, carcinoma, dilatation, chronic gastritis, atony of the stomach.

Retarded motor power of the stomach may be evidenced incidentally by finding in the vomitus or contents removed by tube portions of vegetable or animal food that had been ingested an unduly long time previously. If special examination of the motor power of the stomach is desired, it may be emptied by the tube seven hours after a moderate meal; the presence of food material after that interval indicates sluggish gastric motility. Or easily recognizable food, as boiled rice, may be ingested in the evening, and the stomach emptied by tube, with or without a test meal, in the morning (or later); normally no food should remain that length of time.

Another method consists in administering a gram of salol in three or four capsules immediately after a meal. In the acid gastric juice salol is unchanged, but as soon as it enters the alkaline intestinal contents it is decomposed into phenol and salicylic acid. The latter is immediately absorbed and excreted in the urine, where it may be detected by adding a few drops to a weak aqueous solution of ferric chloride; in the presence of salicylic acid or its derivatives a brown or violet color results. After ingesting the salol the urine should be tested at intervals of half an hour or an hour, and after twenty-four hours. Normally a positive reaction is obtained in sixty to seventy-five minutes; a later result indicates retarded motility, a negative result for over twenty-four hours indicates pyloric stenosis. On the other hand the salol should normally be entirely excreted within twenty-four hours and be absent after that period; if its derivatives are present in the urine after twenty-four or thirty hours retarded gastric motility is indicated. The results will, however, be misleading unless the stomach contents are acid, the intestinal contents are alkaline, and the permeability of the kidneys is normal.

D. EXAMINATION OF ABSORPTIVE POWER OF STOMACH.

A capsule containing about .20 to .40 gram of potassium iodide, all traces of this substance being removed from the outside of the capsule, is swallowed; the saliva is tested for the iodide at intervals of a few minutes by the method given on page

11. In the fasting stomach the iodide normally should be absorbed into the gastric circulation and appear in the saliva in six to fifteen minutes; in various diseases absorption is delayed. The test may be made during both fasting and digestion, absorption being slower in the full stomach.

V. THE FÆCES AND INTESTINAL DISCHARGES.

Examination of the fæces and intestinal discharges affords information as to the state of the digestive and absorptive processes, as to any abnormal conditions of the intestinal tract, and as to the character of the ingesta.

A. COMPOSITION OF THE FÆCES.

The constituents of the fæces in normal and abnormal conditions may be classified as follows:

1. Water (ingested or secreted).
2. Food residue {
 - Indigestible portions of ingested food.
 - Undigested and unabsorbed remnants of digestible food.
 - Fragments of vegetable tissue.
 - Fragments of animal tissue.
 - Amorphous detritus.
 - Fat.
 - Proteids.
 - Carbohydrates.
3. Residue of drugs, etc.
4. Residue of substances secreted and excreted along the alimentary tract:
 - Digestant secretions and ferments.
 - Bile and bile pigments.
 - Mucin.
 - Water.
 - Katabolic excreta (urea, uric acid, xanthin bodies).
5. Substances produced by transformation, decomposition, or fermentation of the primary constituents of the alimentary contents, including:
 - Leucin and tyrosin.
 - Indol, skatol, phenol.

Organic acids.

Gases.

Various other substances.

6. Pathological products:

Water.

Blood.

Pus and leucocytes.

Mucus.

Epithelium.

Fragments of tissue.

7. Calculi.

8. Foreign bodies.

9. Parasites and their ova.

The **water** of the fæces in ordinary conditions consists of the unabsorbed residue of that ingested by the mouth; and in pathological conditions it may be derived in large quantities as a result of secretion or exudation from the intestinal wall from irritant or inflammatory conditions, as in diarrhœa and cholera. The consistency of the fæces depends on the relative proportions of water and solid ingredients. The longer the fæcal matter remains in the intestine the less water is present, it being absorbed into the circulation to greater extent; while the more rapid the action of the bowels the softer and more watery are the passages. Normally the proportion of water ranges from about 60 to 85 per cent, being greater with a vegetable than an animal diet.

Food residue makes up the greater part of the solid undissolved portions of the fæces. It is made up of the indigestible portions of ingested food, and the undigested remnants of digestible food. The presence of an excessive amount of undigested material that is ordinarily digestible indicates one of three things: either (*a*) that the material was ingested in excess; (*b*) that indigestion is present, the digestive or absorptive functions of the alimentary tract being defective; or (*c*) that a lenteric condition is present, that is, excessive peristaltic action which carries the food materials along so rapidly that they have not sufficient time for full digestion or absorption.

The food residue of the fæces may again be divided into the crude food materials, as albumin, starch, and fat, and the products elaborated from these by digestive processes, as peptones, sugar, etc. Presence of the latter and absence of the former

would indicate that digestion was normal, but that absorption was defective.

The chief classes of food residue in the fæces besides water are recognizable fragments of vegetable tissue, recognizable fragments of muscle and other animal tissue, amorphous detritus

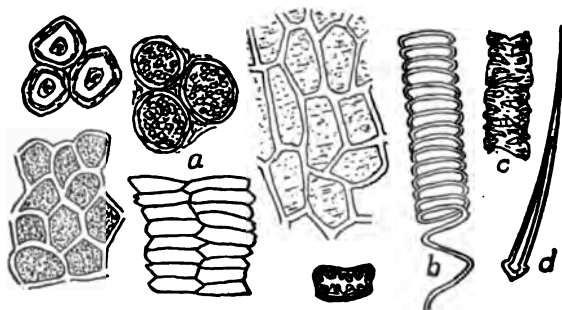


Fig. 18.—Vegetable Tissue in Fæces. a, Vegetable cells and tissue fragments; b, spiral sap duct; c, sap duct; d, hair cell from wheat.

of unrecognizable nature, fat, proteids (especially casein), carbohydrates.

Fragments of vegetable tissue usually and normally occur in the fæces after vegetable diet, varying in character, amount, and size according to the food ingested. The condition of the alimentary organs also influences the amount of vegetable remnants, an excess indicating failure to digest this class of food. These fragments consist chiefly of the indigestible portions of the food (especially cellulose structures), as the stones and seeds of fruits or berries, the rind or skin of fruits (such as apple skins), grains of cereals (like undigested maize grains); sometimes they appear as pulpy masses resembling mucin, but distinguishable under the microscope. Often these fragments are macroscopic in size and recognizable by the naked eye; others are minute, and recognizable only by the microscope.

The microscopical appearance of vegetable tissue (Fig. 18) is very characteristic, so that its recognition is easy. Vegetable cells as a rule are larger than animal cells, and possess thick, prominent walls of cellulose. Often the cells, of a regular polyhedral shape, are uniform in size, shape, and arrangement, the whole exhibiting a regular geometrical pattern. Fragments of spiral sap ducts are often present, and have a characteristic ap-



pearance, that of cylindrical tubes with walls formed by a spirally wound filament; other forms of sap ducts may also be present. Hair cells, especially long, slightly curved, hollow spines from wheat, are very frequent. Starch granules are normally absent, but in abnormal conditions may be present and recognizable.

Fragments of animal tissue are often quite abundant in the fæces after meat diet, and may be increased if proteid digestion is deficient. They occur almost entirely in the form of minute particles of striated muscle fibres; occasionally particles of white fibrous or yellow elastic tissue can be recognized. The fragments are of microscopic size; pieces of meat large enough to be distinguished by the naked eye would occur only in connection with severe alimentary disturbance.

The muscle fragments in the fæces (Fig. 19) are minute oblong or oval fragments, with their corners rounded off by digestive processes, and of a well-marked amber color (from hæmoglobin or stercobilin). In some of the fragments the original cross-striation may still be visible; in others the striation is obliterated by digestion.

Amorphous Granular Detritus.—The largest part of the solid matter of the fæces is composed of finely divided amorphous

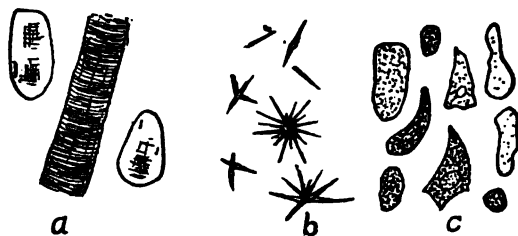


FIG. 19.—a, Remains of muscle fibres in fæces; b, fatty acid crystals; c, degenerated epithelium cells from fæces, especially as mingled with mucus or embedded in mucinous membranes of membranous enteritis.

granular material, the débris of the ingested food. The individual granules are mostly colorless, irregular, and variable in shape and size, mostly ranging from 1 to 5 or 6 micromillimetres in diameter, and the kind of food from which they were derived is usually undeterminable.

Fat.—After ingestion of a moderate amount of fatty food, fat

may be absent from the fæces or present in minute quantity. It is increased after excessive ingestion (as on milk diet), or when from deficiency in the bile or pancreatic secretion or otherwise it is insufficiently absorbed. It is ordinarily demonstrable only by microscopical or chemical methods, but when largely increased its presence may become evident macroscopically, in the form of fatty drops or a grayish, often shiny appearance of the stools. The so-called acholic stools may owe their putty color partly to excess of fat.

Fat occurs in the fæces in the form of ordinary fat, fatty acids, and soaps.

Neutral *fat*, besides its characteristic appearance, may be recognized chemically and microscopically by its low melting point, its solubility in ether, and its staining red with Sudan III.

Fatty acids appear under the microscope as characteristic slender, needle-shaped, or acicular crystals occurring singly or collected together in bundles, sheaves, sectors, or spherical masses, the individual crystals radiating from a common point (Fig. 19). They are soluble in ether, do not stain with Sudan III., and melt with slight heating.

The fatty acids may enter into combination with bases, forming *soaps*, especially calcium and magnesium soaps. These earthy soaps are insoluble in ether, do not stain with Sudan III., do not melt readily, and on treatment with dilute sulphuric acid again yield free fatty acids. Microscopically they appear in amorphous form or as acicular crystals resembling in general those of the fatty acids.

Proteids.—After free ingestion of milk, especially in connection with dyspeptic, fermentative, or diarrhœal conditions, *casein* may appear in the fæces, in the form of small flakes not of great significance, or as abundant large white lumps of curdled milk whose nature is plainly obvious to macroscopic examination and which are of great clinical and therapeutic significance. Casein and fatty flakes may resemble each other and should be differentiated by proper tests.

Ordinary *albumin*, *albumose*, and *peptone* are normally absent from the fæces, but have been encountered in typhoid fever and other intestinal diseases, and may be present in connection with blood or pus. Their recognition has not yet become a matter of clinical importance.

Carbohydrates.—Cellulose is abundant in the fæces as the indigestible residue of vegetable tissues. Starch and its derivatives, the dextrins and sugar, are normally absent, being completely digested and absorbed; in abnormal and pathological conditions they may be present and demonstrable by chemical and microchemical tests.

Drugs.—The unabsorbed residue of drugs may appear in or impart special characters to the fæces. Iron and manganese darken the stools; calomel may turn them greenish; senna, santonin, rhubarb, and gamboge give a yellow color. Bismuth compounds produce a black color, and appear microscopically in the fæces as small, elongated, rhomboid black crystals, or in imperfectly crystalline and irregular black granules. Fatty drugs may cause an excess of fat in the fæces.

The substances secreted and excreted along the intestinal tract, and thus contributed to the intestinal contents, are the various digestant secretions (saliva, gastric juice, pancreatic secretion, intestinal juice), bile, mucin, water, and katabolic excreta (urea, alloxur bodies, etc., in small amount).

The passage of *digestant secretions* into the contents of the alimentary canal may become manifest in the fæces by the presence therein of the various **ferments** of these secretions, namely, the proteolytic ferments pepsin and trypsin (active in acid and in alkaline media respectively), amylolytic or diastatic ferment, milk-curdling ferment, and steapsin. These ferments are normally absent, but in pathological conditions are sometimes present and demonstrable by chemical tests. Besides these, other ferments originate from intestinal bacteria.

Bile enters the intestine in large quantity, but is largely reabsorbed, and the unabsorbed residue is mostly transformed by the time the intestinal contents are expelled.

The proper secretion of bile into the intestine is a matter of so great physiological importance, and deficiencies in this respect are productive of such marked morbid consequences, that the indications afforded by the fæces as to the biliary function are of great clinical importance. The most conspicuous constituent of the bile, and the one that throws most light on the biliary functions, is the bile pigment. Other elements of the bile, biliary acids, cholesterin, etc., entering the intestine are comparatively of little practical clinical importance.

Bile Pigments.—The primary bile pigment, bilirubin, does not normally occur as such in the fæces, the unabsorbed residue being transformed or reduced on its way down the intestine into a yellow pigment, *stercobilin* (identical or closely allied with hydrobilirubin and urochrome), which gives the fæces their yellow or brown color and stains certain of the fæcal constituents amber. The presence of stercobilin is manifested by the macroscopic appearance or by chemical tests.

Abnormally (*a*) the derivatives of the bile pigments may be either absent or diminished in the fæces, or (*b*) unchanged bile pigments may be present.

Absence or deficiency of stercobilin in the fæces is indicated by pale, putty-colored stools, sometimes pasty, sometimes hard and dry, of foul odor, and often containing large amounts of fat. Such dejecta are called “acholic stools,” and result from total or partial lack of bile in the intestine, as from obstruction or hepatic catarrh. The increase of fat is due to its non-absorption through deficiency of bile; and the pale color of the fæces may be due not only to deficiency of stercobilin but also to excess of fat.

Sometimes bilirubin appears in the fæces as such, or changed to biliverdin, without being reduced to stercobilin. Bilirubin and biliverdin give the fæces a yellow and green color respectively. The presence of unreduced bile pigments in the fæces is normal in young infants, but in adults occurs only in abnormal conditions, especially those affecting the small intestine.

The **bile acids** do not ordinarily appear in the fæces as such, being reabsorbed or decomposed in the intestine. *Cholalic acid* formed by decomposition of the bile acids may occur in the fæces. **Cholesterin**, derived chiefly from the bile, rarely appears microscopically in the stools, in the form of its characteristic crystals. *Stercorin* is a substance allied to cholesterin that has been isolated from the fæces.

Mucin and Mucus.—Mucin secreted by the intestinal goblet cells is normally present in small amount in the fæces, but ordinarily in such small masses or so intimately mixed with the other portions of the stool as to be not easily distinguishable.

In catarrhal conditions of the intestine, mucus may be present in the stools in excessive amounts, recognizable macroscopically, microscopically, and chemically. The appearance and admixture of mucus in the fæces vary according to the part of the

intestine from which it is derived; when from the proximal portion of the intestine it is more intimately mixed with the fæces and may be stained with bile; when from the lower part of the bowel it appears in separate masses, colorless; when from low down in the colon it may form a coating on well-formed fæcal masses.

Macroscopically mucus appears in several forms: (*a*) intimately mixed with the fæcal material; (*b*) as separate viscid, tenacious, pulpy masses, colorless or brownish, mingled with fæces or suspended in watery dejecta or irrigation fluid; in cholera as small masses resembling rice grain; (*c*) as a glairy, viscid, often amber-colored coating on the surface of well-formed fæcal masses; (*d*) in mucous enteritis as rather tough, thin, colorless translucent, membranous strips or cylinders, often very long (up to half a metre and more in length), and frequently passed with pain, without accompanying fæcal matter; these membranous masses contain large numbers of granular columnar epithelium cells. Sometimes pulpy vegetable residue resembles mucus, but the distinction can be easily made by the microscope.

Microscopically mucin does not always present a very distinctive appearance; when apparent, especially after treatment with acetic acid, it appears in the form of faint, parallel, straight or curved striæ, sometimes made more distinct by a corresponding arrangement of very fine granular matter. Mucus (especially in membranous enteritis) often contains large numbers of elongated epithelium cells and leucocytes, swollen, granular, and degenerating (Fig. 19).

An important class of substances in the fæces are those produced by the transformation, decomposition, fermentation, or similar changes of the primary ingredients of the intestinal contents. These chemical changes are brought about by the action of the digestive ferments, and in consequence of bacterial growth. A large variety of substances may thus be produced under various circumstances. Some of these are insignificant and unimportant, but others are conspicuous and important. The nature and amount of the end products so produced may afford information as to the occurrence of excessive or abnormal fermentative processes in the intestine.

The following are the chief substances of this class:

Leucin, tyrosin, and other less abundant and less familiar sub-

stances are produced from the hemi-albumose proteids through the action of tryptic digestion. These substances do not ordinarily appear as such in the fæces, but by bacterial action undergo further decomposition, tyrosin forming especially indol, skatol, and phenol. Leucin appears microscopically as yellowish, refractile spherules, sometimes with concentric, sometimes with radiating markings; it is soluble in caustic alkalies, insoluble in ether. Tyrosin appears as clusters of very fine acicular crystals with radial, crossing, or sheaf-like arrangement.

Indol, skatol, phenol, and related substances are produced by bacterial decomposition or putrefaction of proteids; they originate proximately from tyrosin, peptones, etc., and are normal and prominent ingredients of the fæces. They are increased when proteid decomposition is excessive, as after excessive proteid diet, when digestion or absorption is deficient, or when, in constipation or obstruction, the stay of the proteids in the intestine is prolonged. They are diminished in lenteric conditions and in sterile conditions of the intestine, as in new-born infants. In combination with sulphuric acid and potassium they are absorbed and excreted in the urine as ethereal sulphates, and the presence of an excess of these substances in the urine is an indication of excessive albuminous decomposition in the intestines or elsewhere. Indol and skatol give the fæces their fæculent odor, and the intensity of this odor may roughly indicate increase or decrease of proteid decomposition.

Organic acids, of the fatty-acid series (from acetic up to palmitic acid), also lactic and other acids, are ordinarily present, being derived from bacterial breaking up of carbohydrates, fats, and to a certain extent of proteids (as from leucin). These acids contribute to the acid reaction of the fæces. Acid fermentation in the intestine may be excessive, producing markedly acid stools, as in the sour diarrhœas of infants.

Gases in the intestine originate partly from swallowing, partly by diffusion from the blood, chiefly from bacterial decomposition. The most abundant intestinal gases are carbon dioxide, methane, hydrogen, and nitrogen; sometimes ammonia, hydrogen sulphide, and other gases. Excessive discharge of gas indicates excessive intestinal fermentation, while the formation of unusual gases (as NH_3 , H_2S) indicates unusual fermentative processes (ammoniacal fermentation, sulphurous fermentation).

Other substances are sometimes encountered in the fæces. Soluble salts (as sodium chloride) are ordinarily absent or insignificant in amount, being absorbed. Sulphides (as of hydrogen, ammonia, or metallic drugs) may be present. Various pigments coloring the fæces may be derived from certain foods, drugs, or bacterial growth. Insoluble crystalline substances, residuum from food or secretions or formed by chemical reactions, are sometimes present.

Ammonio-magnesian phosphate crystals of characteristic form are frequently present in the fæces, and have been regarded as of little clinical significance; according to Dr. W. G. Morgan they occur quite constantly in cases of hyperchlorhydria and only exceptionally in other conditions.

Crystals of **calcium and magnesium phosphate** are less frequently present. **Calcium-oxalate** crystals, of the typical octahedral form, are not infrequently present after eating certain vegetables. **Calcium carbonate** is rarely encountered, in amorphous form or dumb-bell crystals; it dissolves with the formation of gas on adding acid. **Calcium-sulphate** crystals are rarely present. **Calcium lactate** may be present during milk diet, in the form of crystals resembling those of fatty acids.

Spermin-phosphate crystals (Charcot-Leyden crystals) of the typical elongated octahedral shape are often present in the fæces in cases of intestinal worms (except the trichuris), but are also rarely seen in normal stools and in typhoid fever, dysentery, and pulmonary tuberculosis. Their presence is therefore strongly indicative of helminthiasis.

Blood in the intestinal discharges originates from hemorrhage into the stomach or intestine. Its presence is revealed by macroscopical appearances, microscopical detection of blood corpuscles and pigment, and chemical tests. Albumin and fibrin may also be present. Its condition varies according to its amount and the location of the hemorrhage.

In profuse hemorrhages, even from the small intestine, the blood may pass in large amounts unchanged or in clots, in pure and unmixed form. Unchanged blood is also passed when the hemorrhage, even if slight, is at or near the anus. If the surface of the fæcal masses is simply streaked with fresh blood, the source of the latter is at the anus or vicinity:

When the blood comes from farther up the intestine it (un-

less in large amounts) undergoes change on its way down the canal and is more thoroughly mixed with the fæces. The red corpuscles disintegrate and disappear, the hæmoglobin is transformed to hæmatoidin or other derivatives, and the color changes from bright red to dark red, brown, or black and tarry. As a rule, the farther the seat of hemorrhage from the anus, the greater is the change in the color of the blood and the more intimate its admixture in the fæces. In anchylostomiasis the stools are characteristically dark from altered blood pigment.

Macroscopically, therefore, unchanged and unmixed blood indicates either a profuse hemorrhage or bleeding near the anus. A dark color with the blood well mixed indicates hemorrhage higher up the canal.

Red blood corpuscles distinguishable microscopically indicate either profuse hemorrhage or a low hemorrhage.

Hæmatoidin crystals appear microscopically as minute red rhombic or acicular crystals, often in radiating clusters, or as amorphous granules. They indicate high hemorrhage and transformation of the blood.

In doubtful cases chemical tests may demonstrate the presence of suspected blood.

Pus in unchanged and recognizable form appears in the intestinal discharges chiefly in connection with suppurative conditions of the rectum or lower colon. When originating at a high point, unless in very large quantity (as from rupture of an abscess into the bowel), it becomes so changed and incorporated with the fæces as to be undistinguishable.

Leucocytes appear in the fæces in connection with mucus, blood, and pus, as a result of catarrhal, ulcerative, and suppurative processes. They are normally absent or unrecognizable. If their stay in the intestine is long, they degenerate, becoming granular, and finally disintegrate.

Epithelium cells are usually absent in normal fæces, or if present are very few, scattered, and from granular degeneration and obscuration of the nuclei difficult to recognize amidst the amorphous detritus. In catarrhal conditions they may be present in large number, usually embedded in mucus; they are especially abundant in the mucinous membranous discharge in membranous enteritis. They originate from the columnar epithelium of the intestine, and appear in elongated, elliptical, or swollen forms,

granular and with obscured or invisible nuclei from degenerative changes (Fig. 19). Treatment with acetic acid may clear up the granules, and this or nuclear stains may demonstrate the nuclei.

Fragments of tissue derived from the alimentary tract, such as portions of the mucous membrane or particles of neoplasms, may be present in necrotic, ulcerative, or malignant conditions, and investigation of their histological structure may yield important diagnostic information.

Calculi.—With the fæces may be discharged gall stones, pancreatic calculi, enteroliths, and coproliths. Of these, gall stones are much the commonest, and their detection is of great diagnostic importance. The positive recognition of the source of the calculi is based on their composition and the attendant circumstances. It is very important to distinguish calculi from foreign bodies or undigested masses (as fruit seeds) in order to avoid diagnostic error.

Foreign bodies, such as pins, coins, pieces of bone, resinous masses, fragments of hard vegetable material, and many such objects, are at times passed per rectum, and doubtful objects may cause anxiety, or at least curiosity, until their nature is recognized.

Parasites.—The parasites of the intestine are of two kinds, vegetable and animal.

The **vegetable parasites** include fungi, saccharomycetes, and bacteria. Fungous forms are rarely found in fæces. Saccharomycetes or fungi of the yeast group are common and normal in the fæces; they stain brown with iodine.

Bacteria of many kinds occur normally and in enormous numbers in the fæces, and they with the yeast fungi are the causes of the various intestinal fermentations. Some of the bacteria stain yellow or brown with iodine, others blue or violet.

Pathological intestinal conditions due to bacterial activity may consist in true infectious processes or in abnormal or excessive fermentations. Some bacteria are specific causes of certain diseases, and are present in the fæces only abnormally; other bacteria that are normally present, as the bacillus coli communis, may at times be the cause of abnormal conditions. Among pathogenic intestinal bacteria are the typhoid bacillus, tubercle bacillus, spirillum of Asiatic cholera, bacillus of dysentery, bacillus coli communis, bacillus lactis aerogenes, bacillus proteus vulgaris,

bacillus pyocyaneus, *bacillus* of Finkler and Prior, and various other bacteria capable of causing excessive fermentations and diarrhœas, especially in infants. Among the latter is the green *bacillus* of Le Sage, found in certain infantile diarrhœas, which generates a pigment that gives the fœces a bright green color.

The bacteria of the fœces are investigated by microscopical and cultural methods. The recognition of species of bacteria present would be of great clinical value, but owing to the multiplicity of micro-organisms always found it is in most cases a difficult and tedious procedure.

Animal parasites, in the form of adults, larvæ, or ova, are present in the intestines abnormally, though some of them cause no especial disturbance. The species that have been found in the human intestines and fœces belong to the classes of protozoa, vermes or worms, and insects. Of the parasites that have been observed, many are rare in man, occur in limited and distant localities, or while common in animals only rarely appear in man. Only the commonest and most important forms will be considered (see also Chapter XI.).

In investigating the presence of parasites in the fœces it is important to exclude the possibility of larvæ and other forms being accidentally introduced into the stools after passage.

Protozoa.—The principal protozoa that have been found in the human intestine are the *amœba coli*, coccidia, *cercomonas*, *trichomonas*, *megastoma entericum*, and *balantidium coli*. Of these the *amœba coli* is the most important.

The *Amœba coli* occurs in the discharges from certain forms of tropical dysentery. It is 10 to 20 micromillimetres in diameter, with peripheral clear fluid hyaloplasm and a granular, prominently vacuolated, more central spongioplasm containing the nucleus. The organism exhibits active amœboid movements, the pseudopodia being rounded; the hyaloplasm is the most mobile portion. This amœba is frequently carried to the liver, where it occurs in the abscesses often complicating tropical dysentery.

Cestodes, or tapeworms.—These vermes in the adult or strobila stage consist of a head and neck (forming together the scolex) followed by an indefinite succession of flat segments or proglottids. The head possesses two or four suckers, and on its apex is often a muscular structure, such as a rostellum or proboscis, which may be unarmed, or armed with minute hooks. The lar-

val forms (cysticerci, echinococci, hydatids) infest the muscles or solid viscera. The principal tapeworm found in the United States is the *tænia saginata*; for a list of others found in man see page 242.

Tænia Saginata.—This is at present the only common human tapeworm of the United States, all the other forms being very rare. Infection occurs from eating insufficiently cooked beef containing the larval forms or cysticerci. This worm ranges in length up to 10 metres. The head is about 2 millimetres in diameter, with four suckers, and no rostellum or hooks. The segments or proglottids are flat, rectangular in shape, upward of 1,200 in number, and gradually increase in size away from the head, the maximum being about a centimetre in breadth and

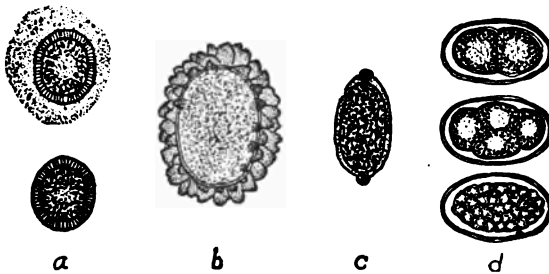


FIG. 20.—Ova of Intestinal Worms ($\times 275$). a, *Tænia saginata*, with and without albuminous covering; b, *ascaris lumbricoides*; c, *trichuris trichiura* (*trichocephalus dispar*); d, *uncinaria duodenalis* (*anchylostoma duodenale*).

up to 2.5 centimetres in length. Sometimes the shape of the segments is atypical, as where they are very short (a few millimetres), but of full width, where they are bead-like, or where the segments are not distinctly marked off from one another. The most conspicuous and distinctive feature of the anatomy of the segments is the uterus, which consists of a central longitudinal portion giving off 17 to 30 branches on each side. When filled with ova the uterus is very conspicuous.

The ova (Fig. 20) are short elliptical or spherical in form, about 30 by 35 micromillimetres in size. Each is enclosed in a conspicuous, thick, translucent, double-contoured vitelline membrane exhibiting fine radial markings; outside of this is sometimes a clear, granular, albuminous envelope. The interior of the ovum is a brown, opaque, granular material containing the embryo in various stages of development.

The ova and segments are passed in the fæces, on which the diagnosis is made. If a large length of the worm is passed, it is important to search for the head to see if it is entirely expelled.

Trematodes.—These are flat or conical worms, common in animals, but rare in man. They inhabit the liver, the cavity of the intestine, or the intestinal wall, from which the ova or worms may pass into the fæces. The following trematodes or ova have been found in the fæces of man: *schistosoma hæmatobium*, *fasciola hepatica*, *dicrocoelium lanceatum*, *opisthorchis felinus*, *opisthorchis sinensis*, *distoma conjunctum*, *fasciolopsis Buskii*, *heterophyes heterophyes*, *amphistoma hominis*, and *paragonimus Westermanii*.

Nematodes, or round worms.—The worms of this class which have been found in the human intestine are: *ascaris lumbricoides*; *ascaris canis*; *ascaris maritima*; *oxyuris vermicularis*; *uncinaria duodenalis*; *strongyloides intestinalis*; *trichuris trichiura*; *trichinella spiralis*.

Ascaris Lumbricoides.—This common parasite is a slender round worm about 4 to 6 millimetres in maximum diameter, tapering to both ends. The head is tri-lobed, the tail pointed and in the male bent at an angle and provided with two spicules. The female is 30 to 40 centimetres long, the male about two-thirds as large.

The ova (Fig. 20) are of an oval form, about 60 by 75 micromillimetres in size. The interior of the egg is a brown, granular material surrounded by a vitelline membrane, and outside this is an irregular, nodular, hyaline, albuminous envelope.

Both the worms and the ova may be present in the fæces, establishing the diagnosis.

Oxyuris Vermicularis.—This common worm somewhat resembles the *ascaris*, but is much smaller, the males being about 4, the females about 10 millimetres long. The worms may be present in the intestine (especially the cæcum) and in the fæces in large numbers, appearing like bits of thread. The ova are about 50 by 25 micromillimetres in size, coarsely granular, and surrounded by a well-marked, doubly-contoured vitelline envelope. The eggs are not ordinarily voided by the worms within the intestine, hence they do not usually occur by themselves in the fæces.

Uncinaria duodenalis (*anchylostoma duodenale*) causes a very severe anæmia, and occurs especially in underground workers.

While commonest in Egypt, Italy, the West Indies, and elsewhere, cases originate in the United States, and in severe anæmias the fæces should always be examined for the ova of this worm. The diagnosis of pernicious anæmia cannot be considered established until such an examination has been made with negative result. The ova are abundant in the fæces, and the worms appear after the use of proper vermifuges. The ova (Fig. 20) are elliptical, 30 by 50 micromillimetres or more in size, and consist of a colorless, thin, distinct vitelline envelope enclosing from one or two to numerous rapidly dividing cells, which are brown, granular, and prominently nucleated. The male adult worm is 6 to 12 millimetres long, with an expanded copulatory pouch and slender penile organ at its posterior end. The female is 10 to 18 millimetres long. The head is turned dorsally and has a hollowed mouth armed with six hooks.

Strongyloides intestinalis is a small worm 1 or 2 millimetres long, which causes diarrhoea. It is common in Cochin China, but has been found in other countries and even in the United States. The female is viviparous, so that the immature worms and not the ova occur in the fæces.

Trichuris trichiura (*trichocephalus dispar*) is a common intestinal parasite and its ova are frequently seen in the fæces, but it causes no troublesome symptoms. The worms are about 4 or 5 centimetres long, and being attached to the intestinal mucosa do not often themselves appear in the fæces. The ova (Fig. 20) are brown and finely granular, covered with a thick, hyaline, doubly contoured vitelline envelope; they are elliptical in shape, with a small, clear, rounded protuberance at each end (a sort of plug filling openings in the shell), and measure about 50 by 25 micromillimetres in size.

Trichinella Spiralis. —The adult forms of the trichina, 1.5 to 4 millimetres long, occur in the intestine. They are viviparous, and the discharged larval forms penetrate the intestinal wall and are carried to the muscles, where they become encysted. In cases of trichinosis the parasites may be found in the stools.

Insect larvæ, as those of *lucilia macellaria* and others, are at times passed with the fæces.

Meconium is the dark, tarry material in the intestine of the fœtus. It is composed entirely of materials secreted into or

given off from the alimentary tract, such as fat, cholesterin, bilirubin, bile acids, various salts, epithelium, etc. Its examination is rarely of diagnostic service.

B. PHYSICAL CHARACTERS OF THE FÆCES.

Amount.—The daily quantity of fæces passed varies with the diet and condition of the intestine, being greater with vegetable food possessing abundant indigestible material, and less with a more completely digestible proteid diet. The normal amount ranges from about 60 to 250 grams per twenty-four hours.

As the water of the fæces is such a variable factor in normal and abnormal conditions, in a precise determination of the daily amount of fæces passed it would be necessary to determine the amount of solids and liquid of the fæces separately.

Consistency.—The consistency of the fæces depends upon the relative amount of solids and fluids present. The consistency is ordinarily sufficiently firm to enable the fæces to assume and maintain definite form. When the water is increased, as in diarrhoeal conditions, the fæces become softer, semi-solid, creamy, or watery. When the water is diminished, as from reabsorption by prolonged stay in the intestine in constipation, the fæces become hard and dry. In some instances the stools are pasty or tarry.

Form.—Soft or fluid fæces are incapable of retaining permanent form. Normal fæces possess the well-known cylindrical shape. Fæces that have been a long time in the intestine may be passed in hard, dry, scybalous balls or masses. Stenosis of the rectum may result in flattened and ribbon-like fæces.

Homogeneity.—Ordinarily the fæces are fairly macroscopically homogeneous, all the elements being evenly mixed together. Large undigested vegetable masses may even normally be present, destroying the homogeneous character of the stools. When the fæcal masses are passed along with a quantity of fluid, or with unmixed mucus, casein coagula, calculi, foreign bodies, worms and the like, the ensemble is heterogeneous.

Color.—The color normally ranges from light yellow to dark yellow, brown, or almost black, depending chiefly on the presence of stercobilin. The greater the amount of water present, the lighter usually is the color. In young infants a green color

from biliverdin is not abnormal; or stools colored yellow with bilirubin may turn green on standing. Milk diet, as in infants, gives the fæces a light yellow color; meat diet produces a darker color, huckleberries and claret a blackish brown. Chlorophyll may give a greenish tinge. Iron, manganese, and bismuth darken or blacken the fæces; calomel may turn them greenish; rheum, senna, and other drugs give a bright yellow.

In pathological conditions the color may have much diagnostic significance. Acholic, putty-colored, or colorless stools are due to deficiency of bile, or excess of fat, or both. Blood mixed with the stools gives a blackish color. In infants a green color may be generated by some of the bacteria causing the diarrhœa.

Odor.—The characteristic repulsive odor of the fæces is due chiefly to indol and skatol, also to volatile fatty acids, gases (methane, hydrogen sulphide), etc. This odor is increased after meat diet, in proteid indigestion, in constipated conditions, or from any similar causes giving rise to excessive proteid decomposition and indol formation. The fæculent odor is less in lenteric and diarrhœal conditions, on vegetable diet, and especially in infants on milk diet, proteid decomposition being less under these circumstances. Excessive acid fermentations produce a sour odor of the fæces, especially in infants. A fetid odor may arise from necrotic processes, as in rectal cancer.

Reaction.—The reaction of the fæces is normally neutral, weakly acid, or weakly alkaline. The acidity is due to the organic acids, the alkalinity to ammonia and other principles. The reaction of the surface of the fæcal masses may be different from that of the interior. Abnormally the reaction may be strongly acid or strongly alkaline, as from excessive acid or ammoniacal fermentations.

C. EXAMINATION OF THE FÆCES AND INTESTINAL DISCHARGES.

The chief methods of examination of the fæces for the determination of the points above considered are macroscopical, microscopic, chemical, and bacteriological.

Macroscopical examination of fæces gives information as to their form, consistency, homogeneity, color, etc., with all that these imply, and enables a sufficiently close estimate to be made of the

relative amount of water present. It reveals the presence of macroscopic masses or fragments of undigested vegetable tissue, fat, coagulated casein, mucus, blood, pus, pieces of tissue, calculi, foreign bodies, and parasites. The presence or deficiency of stercobilin, and the presence of blood or other abnormal coloring matters are also manifested.

The odor may afford some information as to the extent and nature of fermentation processes in progress (proteid, acid, ammoniacal, etc.).

To examine segments of tapeworms as to the morphology of their uterine organs the segments may be flattened out by being pressed between two glass slides, so as to make their internal organs more visible.

To concentrate small parasites, ova, etc., so as to render their detection more easy, the fæces may be thoroughly stirred up with a large quantity of water in a tall cylindrical vessel, and the mixture allowed to settle for a few minutes. The ova and parasites settle very quickly, and the supernatant material can be poured off without loss of parasites. More water is added and stirred up with the residue, the mixture allowed to settle for a minute or two, and the upper portion poured off. This process is repeated a number of times, gradually diminishing the amount of water added, until the fæcal material is largely washed away. The residue containing the parasites may then be spread in a shallow flat dish and the parasites picked out; or it may be examined microscopically.

Microscopical Examination of Fæces.—If the fæces are fluid a representative drop is placed on a slide and covered with a cover-glass; or if hard a minute portion is taken (the bacteriological platinum loop is convenient for the purpose) and mixed with a drop of water on the slide, and the cover applied. The specimen thus made reveals the following under the microscope so far as they are present: small fragments of vegetable tissue, chlorophyll and starch granules, particles of muscle or fibrous tissue remaining from the food, a large amount of amorphous and granular detritus, fat, fatty-acid crystals, bismuth crystals, cholesterol crystals, mucus, ammonio-magnesian phosphate crystals, calcium and magnesium phosphate, calcium oxalate, calcium carbonate, spermin-phosphate crystals, hæmatoidin, red blood corpuscles, leucocytes, epithelium cells, fragments of intestinal

tissue, saccharomycetes, bacteria, small animal parasites or their ova.

Special treatment is necessary to aid in the recognition or demonstration of some of the microscopic bodies. The addition of Sudan III. solution (page 10) stains particles of fat red and so makes them obvious. The addition of iodine solution (page 11) demonstrates any starch granules that may be present by coloring them blue; iodine also gives cells a yellow color, and stains saccharomycetes and some bacteria brown, others blue. The addition of acetic acid may make leucocytes and epithelium cells more distinct by clearing the granules from their cytoplasm and making the nuclei more prominent; acetic acid also makes mucin more distinct, dissolves any phosphatic crystals present, and clears up carbonate crystals with the evolution of bubbles of gas. Cells may also sometimes be demonstrated by the use of the ordinary nuclear stains, dried cover-glass films being prepared, fixed, and stained.

Bacteria may be brought out by the usual staining methods; the presence of the tubercle bacillus can sometimes be demonstrated by the use of the specific stains for this bacillus, as Gabbet's method. To examine for *amœba coli* the stool should be fresh, passed in a warmed receptacle, and examined on a warm slide so as not to check the amœboid movements. Fragments of tissue may be examined extemporaneously by teasing or otherwise, or by hardening and sectioning in the usual manner. Foreign bodies, seeds, vegetable fragments, and the like may be crushed or teased until fine enough for microscopical examination. Pulpy vegetable masses may be distinguished from mucus by microscopical examination, the former exhibiting vegetable structure.

Mucin may sometimes be demonstrated by its staining reactions, which are of a modified basophilic nature; after proper fixation (alcohol, mercury bichloride, or heat) it stains blue with methylene blue, green with the triple stain, reddish with toluidin blue (1-per-cent solution in 5-per-cent phenol).

Fibrin stains red with the triple stain, blue by Weigert's gentian-violet method.

Cellulose stains yellow with iodine solution, and then turns blue after being quickly washed and allowing pure sulphuric acid to flow under the cover-glass.

Chemical Examination of Fæces is only occasionally required to determine as to the presence of special substances, not having as yet been extensively elaborated for clinical application. A number of micro-chemical tests have been already given.

The reaction may be taken by litmus paper. If the fæces are hard and dry, the paper should be moistened, thrust into the fæcal mass for a time, and the adherent fæces then washed off. A quantitative estimation of the degree of alkalinity or acidity may be made by titrating a watery extract of a known amount of the fæces with decinormal acid or alkali.

The amount of water and solids may be determined by weighing before and after drying.

In exact investigations of metabolism the total nitrogen of the fæces may be determined, as by Kjeldahl's method.

Fat may be recognized qualitatively by its solubility in ether or its reaction with Sudan III. Fat and fatty acids can be extracted with ether and thus estimated. Insoluble soaps may be decomposed by digestion with dilute sulphuric acid, and the separated fatty acids extracted with ether.

Proteids may be extracted by digesting the fæces in water acidulated with acetic acid, and then filtering. The ordinary tests for albumin, albumose, and peptone are then applied to the filtrate.

To test for carbohydrates, boil the fæces with water, filter, concentrate the filtrate by evaporation, and test it for starch and erythro-dextrin with iodine, and for sugar by the usual tests.

The ferments, proteolytic, amylolytic, or milk-curdling, may be tested for by treating coagulated proteid, starch solution, or milk with acid or alkaline watery extracts of the fæces.

Stercobilin is ordinarily indicated by the color of the fæces. To determine if the lack of color of colorless stools is due to excess of fat or lack of stercobilin, extract the fat with ether; if stercobilin is present the residue will assume a yellow color.

Bilirubin may be tested for by adding a drop of nitroso-nitric acid to the fæces, or by testing a watery or chloroform extract in the regular way, a green color indicating the presence of bilirubin.

Mucus particles may be tested by hardening them in alcohol or mercuric bichloride and then staining with the triple stain; mucin stains green, fibrin red.

Blood may be tested for by drying a small portion of the suspected material and applying the hæmin test, or by applying the guaiacum test to a watery extract.

Calculi: In searching for suspected biliary or other calculi, the fæces should be stirred with water and washed through a sieve. The material thus obtained (in the form of small particles or large concretions) may then be examined chemically as to its nature.

Bacteriological Examination of the Fæces.—Such a large number of bacteria of so many different kinds occur in the fæces that it is difficult to isolate and determine those present, and in most cases is utterly impracticable with the time and labor available for clinical purposes, although their recognition would be of great service clinically.

Tubercle bacilli can sometimes be demonstrated by their special staining reactions; care should be taken to exclude smegma bacilli. Methods have been presented for the detection of typhoid bacilli in the fæces, as those of Elsner and Piorkowski, but have not come into general use. In cases of cholera Asiatica the comma bacilli may be so predominant as to be readily separated.

VI. THE SPUTUM.

The sputum is an abnormal product altogether, and indicative of disease of the respiratory system or adjoining organs. The object of its examination is to aid in determining the nature and seat of the disease.

The amount of sputum expectorated is very variable in different cases, ranging from a few cubic centimetres up to 500 or more in twenty-four hours. Aside from temporary conditions like pulmonary oedema and hemorrhage, or rupture of abscesses, continuous profuse expectoration, serous or purulent, may occur in bronchorrhœa, bronchiectasis, chronic bronchitis, phthisis, etc., and causes a severe drain on the vital energies.

Composition.—The chief elements entering into the formation of sputum are: Serum, mucus, pus, blood, air, pigment, putrefactive products, parasites (vegetable and animal), leucocytes, red blood corpuscles, epithelium, fragments of tissue, Curschmann's spirals, Dittrich's plugs, fibrin, crystals, calculi, foreign bodies.

The serous or watery constituents of the sputum originate from oedematous transudation, inflammatory exudation, or hypersecretion, thus being expectorated in large amount in pulmonary oedema and bronchorrhœa.

The mucus of the sputum originates from catarrhal inflammation, and contributes to the expectoration its viscid tenacious consistency. It is recognizable macroscopically and (by its form and staining reactions) microscopically. It is usually light and buoyant, and floats in water.

The purulent constituents of the sputum originate from suppurative processes. Sometimes sputum has the creamy consistency of pure pus; but in the suppurative catarrhal conditions common in the respiratory passages the pus is intimately mixed with mucus, giving the sputum a mingling of the characters of the two. The proportion of pus present is measured by the abundance of leucocytes observed microscopically, their number being less the less is the pus and the greater the amount of mucus

and other materials mingled with the pus. Pure pus usually sinks in water.

Blood in the sputum arises from congestive and hemorrhagic conditions. If present it may range in amount from slight streaks of blood in the sputum, through mixtures of blood with serum, mucus, or pus, to pure blood. It is recognized by its color, by the presence of red blood corpuscles microscopically, and if necessary can be tested for chemically. When expectorated freshly shed it is bright red in color, venous blood being darker than arterial; but after remaining in the lungs for a time it turns dark, or the hæmoglobin may undergo transformation to dark or brown pigments (as in the rusty and prune-juice expectoration of croupous pneumonia).

Air may or may not be mixed with the sputum, forming a frothy, foamy, or bubbly mass; and sputum when put into water sinks or floats according to its air content. It is more abundant the smaller the bronchi in which the sputum is generated; and is especially scanty in sputum originating from phthisical cavities or large bronchi.

The main substance of the sputum is ordinarily formed of serum, mucus, pus, and blood; any one of these may predominate, or two or more may be mixed in varying proportions, and air may be present or absent. Sputum owes its consistency and form to these ingredients, and according to the predominating constituents specimens of sputa can be described as serous, mucoserous, mucous, muco-purulent, etc. The relative proportions of the various ingredients indicate the relative intensity of the various processes (catarrhal, suppurative, etc.) that generate mucus, pus, serum, or blood respectively.

The **consistency** of sputum depends on its predominating ingredients; if serous, it is thin and fluid; if mucous or muco-purulent, it is tough, viscid, and tenacious; if purely purulent it is creamy. A muco-serous variety of sputum is frequently seen, containing large numbers of bacteria and abundant squamous epithelium, but very few leucocytes, that is of creamy consistency.

Form.—The chief types of sputum are serous, mucous, mucoserous, purulent, muco-purulent, and bloody.

The purely serous, mucous, and purulent sputa, sputum of pure blood, many muco-purulent sputa, or any sputum in which

the ingredients are thoroughly commingled, are homogeneous. Combinations of mucous or purulent elements with serum are usually heterogeneous, the masses of mucus or pus being suspended separate in watery fluid. The so-called nummular sputum consists of small rounded or caseous masses sinking in serum; this is ordinarily stated to be characteristic of phthisis. The rusty sputum of croupous pneumonia is a viscid muco-pus colored brown by altered blood pigment. The prune-juice sputum of the same disease is a serous sputum stained dark by transformed hæmoglobin.

Lamination.—When abundant and thin, sputum on standing often separates into layers, which may be worthy of note. Three layers usually form; the upper stratum is formed of the buoyant air-containing frothy portions (mostly mucus); the middle stratum consists of the serous fluid elements; the lowest stratum is a sediment of cellular elements, tissue fragments, and granular débris.

Color and Pigment.—Purely serous and mucous sputa are ordinarily colorless and hyaline. Other sputa are colored from various pigments. Purulent sputum and sputum containing sufficient purulent elements are yellow or green. A green color may be imparted to the sputum by pigments generated by bacteria, by biliverdin in cases of jaundice and rupture of the liver into the lung, and sometimes in cases of pulmonary sarcoma and carcinoma. Dark-colored sputum, containing melanin, is sometimes expectorated in cases of melanotic tumors of the lungs. Blue and bright-yellow sputa have been observed, due to bacterial pigments, or to altered bile and blood pigments. Peculiar colors sometimes develop (from bacterial growth) after the sputum has stood some time after being expectorated.

The presence of blood or the derivatives of hæmoglobin, in hemorrhagic and congestive conditions of the lungs, gives sputum a color varying from bright-red to brown, according to the time elapsing between the extravasation of the blood and its final expectoration. When expectorated freshly shed the color is bright red; when longer retained before being spit out, it turns first darker and then brown or rusty, as in pneumonia, infarction, brown induration.

In pneumoconiosis, or the free inhalation of extraneous particles of dust of various kinds in certain occupations, the sputum

is colored corresponding to the dust inhaled. The particles most frequently inhaled are of carbon, giving the sputum a black color. Particles of iron oxide give a dark or reddish color; ultramarine produces a blue sputum in workers in this substance; other dusts similarly inhaled are silicious, calcareous, metallic, etc.

Putrefactive products are generated in necrotic or foul suppurative conditions or where the sputum is retained a long time in the cavities or pulmonary passages before being expectorated, and so undergoes decomposition.

The **odor** of fresh sputum often is slight and not markedly disagreeable; but in connection with putrefactive conditions it is intensely foul, fetid, putrid, and repulsive.

The **reaction** of fresh sputum is usually alkaline.

Parasites.—The following **animal parasite** forms have been observed in the sputum, but all are rare in this country:

Amœba coli occurs in cases of amœbic hepatic abscesses extending or rupturing into the lungs. *Trichomonas*, *paramecium*, and other protozoa have been observed, especially in putrid pus or gangrene. Hooklets, portions of cyst membranes, or even entire hydatid cysts may occur in the sputum derived from the larval cysts (hydatids) of the *tœnia echinococcus* developed in the lungs or liver. The larval cysts of the *tœnia solium* (cysticercus cellulossæ) at times develop in the lungs. *Paragonimus Westermanii* is a trematode infecting the lungs and causing hæmoptysis; it is common in eastern Asia, but a few cases in animals have been observed in the United States; ova in great numbers and occasionally the entire worm may occur in the sputum, establishing the diagnosis. The ova of *schistosoma hæmatobium* have been known to appear in the sputum.

Vegetable Parasites.—Fungi exceptionally occur in the sputum, as actinomyces, streptothrix, aspergillus, mucor, oidium albicans, saccharomyces, leptothrix, and others, some pathogenic (causing pneumomycosis), others innocuous or secondary to tuberculous or other lesions. In pulmonary affections caused by fungi, but simulating tuberculosis, instead of tubercle bacilli the mycelia of aspergillus, mucor, or streptothrix, etc., are found in the sputum. The most numerous and important of the micro-organisms of the sputum are the **bacteria**; many of these are non-pathogenic and harmless, while others are pathogenic and of the utmost diagnostic import.

The most important pathogenic bacteria are the tubercle bacillus, micrococcus lanceolatus, bacillus influenzae, streptococcus pyogenes, staphylococcus pyogenes, micrococcus tetragenus, Friedländer's bacillus.

The tubercle bacillus appears in numbers varying from a few to many in pulmonary tuberculosis, and its detection is of the highest importance in the diagnosis of that disease. Failure to find it does not, however, necessarily negative the diagnosis of tuberculosis unless after many repeated examinations. It is usually associated with a relatively purulent type of sputum, containing many leucocytes, few squamous epithelium cells, and few other bacteria. The tubercle bacillus is a long, slender, rod-shaped bacterium, usually with a beaded appearance.

Micrococcus lanceolatus, the diplococcus of croupous pneumonia and other diseases, is observed in the sputum in large numbers in that disease, though it may be present in healthy individuals. It is an oval micro-organism, broader at one end than the other, arranged in pairs, end to end, the broad ends toward each other; sometimes it forms short chains, and in the sputum and blood is usually surrounded by a capsule. Its presence in the sputum is significant of pneumonia only in connection with the clinical symptoms.

The **bacillus of influenza**, a very small, slender bacillus, single or in pairs, is observed in the sputum in that disease.

Other bacteria, pathogenic or innocuous, of numerous varieties, and in large numbers or dense aggregations, are frequently present in the sputum, such as streptococci, staphylococci, micrococcus tetragenus, sarcinae, etc.

Leucocytes are present in sputum in numbers proportional to the amount of pus present. The greater the amount of mucus or serum in the sputum, the fewer and more scattered are the leucocytes; while their number increases and they may be exceedingly numerous as the sputum approaches a purulent type. Their number is therefore an index of the extent and degree of the suppurative process present.

In ordinary suppurative cases the leucocytes are almost entirely of the polynuclear variety. In some cases, especially asthma, eosinophile leucocytes may be abundant and even preponderate.

Red blood corpuscles, either fresh or more or less altered, are observable in bloody sputum.

Epithelium cells detached from the respiratory passages frequently appear in the sputum in moderate numbers, in their normal or a more or more or less altered condition: There are three types of epithelial cells along the normal respiratory passages, large squamous cells in the mouth and pharynx, ciliated columnar cells in the nose, larynx, trachea, and bronchi, and "alveolar cells" in the air vesicles. In catarrhal conditions and after remaining a time in the sputum these cells may undergo changes so that the site of their origin cannot be determined.

Squamous cells, large and flat, are the form most frequently present in sputum. They come from the mouth, pharynx, or upper part of the larynx, and are often numerous in the mucoid sputa, associated with relatively few leucocytes and with large numbers of bacteria. In more purulent sputa they are less numerous.

Ciliated columnar cells, so little altered as to be recognizable as such, are rarely seen in the sputum; they may come from the bronchi or trachea, but are more apt to come from the nose.

Rounded or Oval Epithelium.—Swollen, rounded, or otherwise altered epithelium cells are frequently present mingled in small numbers with the leucocytes, sometimes showing granular or fatty degeneration. They originate from catarrhal surfaces, being immature or germinal cells prematurely detached, or from columnar or flat cells that have become swollen or altered by the action of the sputum. Such cells, which are termed "mucous corpuscles" or "mucocytes," are usually spherical in form, larger than leucocytes, with a single large, round nucleus and granular cytoplasm.

Alveolar Cells.—The pulmonary alveoli are lined with flat squamous cells intermingled with spheroidal cells. The flat squames do not appear in the sputum as such, but if present at all swell to a spheroidal form. The nature of the so-called "alveolar cells" of the sputum is not definitely settled, but they are supposed to originate in the alveoli of the lungs, perhaps from the spheroidal cells. The alveolar cells are 20 to 50 micromillimetres in diameter, oval in form, with one or sometimes more than one rounded nucleus; the body protoplasm is finely granular, and is often studded with granules either of extraneous matter, fat, myelin, or pigment derived from hæmoglobin.

Sometimes one and the same cell may contain more than one kind of these granules. The epithelial cells from other parts of the respiratory tract rarely show the pigmentation or the fatty or myelin degeneration exhibited by the alveolar cells.

The **extraneous granules** that may infiltrate the bodies of the alveolar cells are particles of dust, most frequently consisting of carbon, inhaled in pneumoconiosis and taken up by the cells. The globules of **fat** that may be present originate from fatty degeneration of the cells. Often the cells exhibit, from degenerative changes, irregular, often large particles of a clear, fat-like, refractile, pale material, sometimes concentrically marked; this substance is regarded as a form of **myelin**. The cells are sometimes almost broken down into this material, and rounded masses of myelin may appear free in the sputum. Bodies similar in appearance to corpora amylacea, and also resembling myelin drops, are said to appear sometimes in the sputum, turning blue with iodine.

In cases of prolonged congestion or brown induration of the lungs, especially from mitral disease, but also in infarctions and pneumonia, the alveolar cells may contain numerous particles of brown or yellow pigment, derived from hæmoglobin. Such cells are called **heart-disease cells**, and are of diagnostic significance.

The alveolar cells are of common, almost constant, occurrence in the sputum, being present in small numbers in the slightest respiratory affections, as well as in large numbers in more serious conditions like phthisis. They have little or no diagnostic significance except in the case of those containing altered blood pigment (heart-disease cells), which indicate stasis or extravasation of blood.

Carcinoma Cells.—Epithelial cells of various forms derived from pulmonary carcinoma may appear in the sputum, and in two or three instances the diagnosis of cancer of the lung has been so made. It is, however, difficult to identify isolated cells, not in the typical alveolar arrangement, as with certainty coming from carcinomatous growths, except perhaps when abundant and constant and associated with corresponding clinical symptoms.

Fragments of tissue are at times expectorated, such as macroscopic pieces of lung substance, pieces of cartilage, and especially elastic fibres (separate or in an alveolar network), in necrotic

conditions like pulmonary gangrene and phthisis. In two or three instances malignant disease (sarcoma) of the lung has been diagnosticated from fragments of the tumor expectorated; but this is only rarely possible.

Curschmann's spirals are small, elongated, rather firm bodies up to about 1 or 2 centimetres in length and 1 millimetre in diameter, composed of a sort of mucinous, fibrillar material twisted spirally around a central, often sinuous filament. They are more or less opaque, pale in color, sometimes embedded in hyaline material. Forms varying from the type may occur, like branching forms and those lacking the central filament. They are sometimes studded with epithelium cells, leucocytes, or Charcot-Leyden crystals. The spirals occur in certain forms of asthma and at times in other cases.

Dittrich's plugs are opaque, whitish-yellow particles, foul in odor, in size from that of a millet to a mustard seed, occurring in sputum in putrid conditions (gangrene, foul bronchitis), and consisting of masses of bacteria mingled with fatty-acid crystals. They originate in small bronchi or tonsillar crypts.

Fibrin, in the form of amorphous flakes or (rarely) of casts of the bronchi, may be expectorated in croupous pneumonia and the rare cases of croupous or fibrinous bronchitis. In the latter condition large casts of the ramifying bronchial passages are sometimes expelled.

Crystals of various kinds are exceptionally present in sputum. The most significant are the *Charcot-Leyden crystals*, apparently crystals of spermin phosphate, which are small, greatly elongated and slender octahedral crystals, occurring chiefly in some cases of asthma. *Hæmatoidin* crystals or flakes sometimes appear in sputum after hemorrhagic extravasations. Fatty-acid crystals are occasionally encountered. Crystals of cholesterin, leucin, tyrosin, soaps, calcium oxalate, calcium carbonate, and ammonio-magnesian phosphate have been observed, especially in putrefactive conditions.

Calculi from the bronchi or lungs have been expectorated in rare instances.

Foreign bodies, as pieces of bone, that have entered the air passages are occasionally coughed up. Particles of food from the throat or mouth frequently become mixed with sputum, and should not be allowed to mislead the observer; particles of ani-

mal food, for instance, should not be erroneously regarded as indicating pulmonary necrosis.

EXAMINATION OF SPUTUM.

Sputum is chiefly examined macroscopically and microscopically; exceptionally occasion may arise for its examination bacteriologically (by cultures or animal inoculation) or chemically (as for traces of blood). The sputum expectorated early in the morning before breakfast is advantageous for examination, being usually abundant, representative, and unmixed with food particles. In the case of thin fluid sputum, the essential elements should be collected by allowing them to settle or by using the centrifuge, by which the sediment may be obtained.

Macroscopic Examination of Sputum.—Simple inspection reveals much information as to the character of the sputum, its content of serum, mucus, pus, blood, and air, its consistency and form, color, odor, etc., with all that these signify. In cases of profuse expectoration, the amount discharged in twenty-four hours, to be determined by measurement, is a useful clinical datum.

To make a comprehensive search of a quantity of sputum for objects like fragments of tissue, etc., it may be pressed out into a thin layer between two sheets of glass, when suspicious portions may be readily picked out and further examined; the use of a dark background aids in the examination.

Microscopical Examination of Sputum.—The most important diagnostic data to be obtained from the sputum are afforded by microscopical examination. Sputum may be examined either fresh or after being appropriately stained. In examination in the fresh condition a small quantity is placed on a slide and a cover-glass applied, when it is ready to be placed under the microscope.

In staining sputum it is necessary first to spread and fix it, which is mostly done by the same process for the different methods of staining. A small portion of suspicious parts of the sputum is taken on the point of thumb forceps or with a platinum loop, and placed on a clean cover-glass. Another cover-glass is then placed over the drop of sputum, and the two covers are pressed together between the thumb and forefinger or between the points of forceps, so that the sputum spreads out in a thin layer between the two cover-slips. The two slips are then slid

apart and separated, leaving a thin film of sputum on one side of each. The films are then dried at the room temperature, or by gentle warming high above a flame; if held in the fingers above the flame there is no danger of overheating.

The films of sputum can also be spread by means of the wire loop on a glass slide or single cover-glass.

The films being prepared and dried, they are next to be "fixed," preparatory to staining. For staining bacteria and most other purposes this is done by heating or "flaming" the specimen, holding it by forceps and passing it at medium speed through the flame of a Bunsen burner or alcohol lamp three times, at brief intervals.

Examination for Tubercle Bacilli.—This is the object for which the great majority of sputum examinations are made. The specific method usually employed for demonstrating these bacilli depends on the fact that after being well stained by fuchsin they retain this stain even when treated by strong acids that decolorize all the other objects in the specimen. There are two or three steps in the process, staining with fuchsin, decolorizing with acid, and counterstaining.

The fuchsin solution ("carbol-fuchsin") is prepared as follows: Two stock solutions are kept on hand, a 5-per-cent aqueous solution of phenol, and a saturated solution of fuchsin in alcohol. The staining solution is prepared by mixing 9 parts of the phenol solution with 1 part of the fuchsin solution; this solution deteriorates after a few weeks, and hence should be freshly prepared from the stock solutions at proper intervals.

To stain, the cover-glass preparation, fixed by heat as above described, is held in self-retaining (Fig. 13) or thumb forceps and covered with as much of the carbol-fuchsin solution as it will hold. It is then heated carefully over a Bunsen burner, until the fluid just boils (bubbles) two or three times. After a minute or two the staining fluid is washed off with water.

The specimen is next decolorized by immersing it in a 25-per-cent solution of nitric or sulphuric acid in water, for ten to sixty seconds, until the red color is just discharged and the film just decolorized. It is then immediately washed with water. It is then counterstained with Löffler's methylene-blue solution, washed, and mounted temporarily on a slide with water, the upper surface of the cover-glass dried with filter paper, and ex-

ained with an oil-immersion objective. Permanent mounts can be made with Canada balsam.

When thus prepared, the tubercle bacilli appear conspicuously, stained red, while other bacteria and cell-nuclei are stained blue.

Gabbet's method: For routine work Gabbet's method is very largely used, being substantially the same as that just described, but with the decolorization and counterstaining combined in one process. The specimen is spread and fixed in the usual manner, and stained by carbol-fuchsin in the manner described. After being washed with water the cover-glass is covered with Gabbet's solution, consisting of 75 parts of water, 25 of sulphuric acid, and 2 of methylene blue. This is allowed to remain for fifteen to sixty seconds, until by washing and examining against a white background the red color is discharged and a pale blue appears. The specimen is then washed and mounted as before. Tubercle bacilli are stained red, nuclei and other bacteria pale blue. If a more decided counterstaining is desired, Löffler's methylene blue can be used after Gabbet's solution.

When the tubercle bacilli are very scanty and difficult to demonstrate, it may in doubtful cases be advisable to adopt means to facilitate their detection.

One method is by sedimentation. To make the sputum sufficiently fluid and remove the cellular elements it is boiled with three or four times its volume of water together with a few drops of liquor sodæ, and the sediment obtained for examination either by centrifugation or after allowing it to settle for twenty-four hours. Or the sputum may be mixed with several times its volume of water, made alkaline, a small amount of commercial pancreatin added to digest away the cellular elements, and the whole set away at a temperature of 87° C. in a conical glass for twelve to twenty-four hours; the sediment is then examined for the bacilli (and elastic fibres).

By placing the sputum in an oven at 87° C. for a day or two the bacilli may increase in number so as to be more easily found. Or by injection of the suspected sputum into a guinea-pig the presence of tubercle bacilli may be shown by the lesions developed after three or four weeks.

In the great majority of cases of pulmonary tuberculosis the bacilli may be easily found at every examination. Failure to find the bacilli must not, however, be taken to negative the diagnosis of tuberculosis, especially if only one or a few examinations are made. A probable negative diagnosis can be arrived at if the bacilli are not found after many trials repeated at various stages of the disease and with specimens of sputum and during

periods in which ordinarily the bacilli would be expected to appear.

Examination for Other Bacteria.—Bacteria other than the tubercle bacillus are ordinarily shown with sufficient distinctness after Gabbet's or other tubercle-staining process has been applied, and even (especially fungi) in unstained specimens. They are not ordinarily of sufficient importance to require special staining, but if it is desired to demonstrate them carefully, the cover-glass preparations, duly spread and fixed, may be stained by the usual bacterial stains, as Löffler's methylene blue, weak carbol-fuchsin, gentian-violet, or Gram's method.

The *micrococcus lanceolatus*, or pneumococcus, is next to the tubercle bacillus in clinical importance so far as the sputum is concerned, and may at times require careful examination by methods which bring out the capsule. Gram's method of staining is very satisfactory; this demonstrates the capsule nicely and does not decolorize the diplococcus. The bacterium is often well shown even after Gabbet's method.

The *bacillus influenzae* may be stained by the usual stains, especially Löffler's methylene blue or a solution of carbol-fuchsin diluted to a pale-red color; it decolorizes by Gram's method. Examination for this bacillus is not in general use for clinical purposes.

The cells of various kinds in the sputum are ordinarily sufficiently evident in the specimen stained for tubercle bacilli. If it is desired to study them especially, the sputum may be examined microscopically in the fresh, moist, unstained condition, using dim illumination; the addition of acetic acid may clear the cells and make the nuclei more evident. Or the cover-glass preparations may be stained by appropriate methods, as with hæmatoxylin-eosin or by the triple stain (to demonstrate the varieties of leucocytes). *Mucin* in stained specimens appears as streaks of amorphous matter, staining with basic stains; *fibrin* might be demonstrated by its oxyphile staining reactions.

Tissue fragments may be teased or embedded and sectioned, and thus stained. *Elastic fibres* may be examined unstained by pressing suspicious particles of sputum out under the cover-glass into a very thin layer, so as to render them visible in the mucus. If a careful search for elastic fibres is needful, the sputum may be boiled with an equal amount of 10-per-cent solution of potas-

sium or sodium hydrate, then diluted with four times as much water. After allowing it to settle twenty-four hours or using the centrifuge, the sediment is examined for the fibres. If found, especially in an alveolar arrangement, the existence of a destructive process is demonstrated.

Crystals, myelin drops, protozoa, fragments of echinococci, ova, etc., may be examined for in the fresh unstained state of the sputum. Fat may be demonstrated by Sudan III.

VII. THE URINE.

Examination of the urine in many cases affords information of great clinical and diagnostic value, if not positively at least negatively. Containing as it does a large part of the waste products of metabolism, especially nitrogenous metabolism, the urine is a valuable index of the state of the metabolic processes in general; both on the anabolic or assimilative side (as when it contains substances that normally are completely elaborated into protoplasm, but owing to abnormal conditions are arrested in an intermediate stage and so excreted), and on the katabolic side (as when unusual products are formed by perverted katabolism and so excreted); it reveals the existence of abnormal conditions of the urinary organs; and in special cases affords information as to the local processes in other parts of the body besides the urinary tract, or as to food, poison, or adventitious substances introduced into the system.

A. COMPOSITION OF THE URINE.

The constituents of the urine consist of liquids, solids, and gases in solution, and particulate or undissolved solid elements (either crystalline, organized, or amorphous). They originate chiefly from the waste products of metabolic activity, as a result of local or distant pathological conditions, or from the surplus or unused portions of ingested alimentary or drug materials. The chemical elements represented in the urinary materials are: among the acid-forming and non-metallic elements, oxygen, sulphur, nitrogen, chlorine, phosphorus, carbon, silicon, and hydrogen; traces of iron, and the alkaline and earthy bases potassium, sodium, ammonium, calcium, and magnesium. The individual substances composing the urine are very numerous, of which some are abundant and conspicuous, while many are minute in amount, obscure in nature, difficult to demonstrate, of inconstant or rare occurrence, and, usually, of no clinical importance.

The substances entering into the formation of the urine in normal and abnormal conditions are as follows:

Water.

Nitrogenous bodies:

Urea.

Alloxur bodies: xanthin bases, uric acid, urates, allantoin.

Hippuric acid; benzoic acid.

Creatin and creatinin.

Leucin and tyrosin.

Sulphur compounds: sulphates, mineral and conjugate; unoxidized sulphur compounds.

Phosphoric acid and phosphates.

Carbon dioxide and carbonates.

Chlorides.

Calcium oxalate.

Pigments and chromogens.

Fat, fatty acids, soaps, lactic acid, other organic acids, ferments, hydrogen dioxide, iron, ammonia, oxygen, nitrogen.

Proteids: nucleo-albumin, serum-albumin, globulin, albumoses, fibrin.

Carbohydrates: glucose, levulose, inosit, sucrose, lactose, maltose, pentoses, glycogen, dextrin, animal gum.

Glycuronates.

Alkapton.

Acetone, diacetic acid, β -oxybutyric acid, alcohol.

Blood and derived substances: proteids, red blood corpuscles, leucocytes, hæmoglobin, methæmoglobin, hæmatin, hæmatoidin, hæmatoporphyrin, and other derivations of hæmoglobin.

Lymph.

Pus: leucocytes, proteids.

Bile: bilirubin, bile salts, cholesterin, nucleo-albumin.

Substances producing the diazo reaction.

Toxins.

Adventitious ingesta.

Epithelium.

Fragments of tissue.

Leucocytes.

Spermatozoa.

Casts.

Cylindroids.

Mucous threads.

Granular and amorphous debris.

Calculi.

Parasites, animal and vegetable.

Foreign bodies.

Water is the most abundant and one of the most important constituents of the urine, and one to which too little consideration is frequently given in clinical work. The amount of water determines the quantity of the urine and its degree of concentration or dilution, and its specific gravity and proportion of solids are intimately dependent on the amount of water. The water is very variable in amount according to circumstances, ranging ordinarily from about 1,000 to 1,500 cubic centimetres daily.

The water is increased, causing a diluted condition of the urine, or hydruria, after copious ingestion of fluids, decrease of perspiration, from the action of hydragogue diuretics, in diabetes insipidus and mellitus, in certain neurotic conditions, in chronic interstitial nephritis. Water is decreased when little fluids are ingested, when much water is lost by free perspiration, catharsis, or vomiting, in chronic parenchymatous and acute nephritis, in febrile conditions, in yellow fever, in cirrhosis, and acute yellow atrophy of the liver, etc.

Urinary Solids.—The total daily amount of solids excreted in the urine is more constant than the water and of much clinical importance. In some cases the amount of solids varies to a certain extent with that of the water, but ordinarily the wide fluctuations of the water are entirely independent of the solids, and the amount of the latter must be determined and considered entirely apart from the amount of water. The specific gravity of urine and the percentage proportions of any of its constituents do not in themselves alone convey sufficient information for clinical purposes, since the amount of water is so variable; but when taken in connection with the daily amount of urine passed they afford accurate and adequate information as to the daily amount of solids excreted, and hence as to the condition of the vital and metabolic processes that produce the excreted solids and as to the efficiency of the organs concerned in their excretion.

The normal amount of solids daily excreted in the urine is about 55 to 75 grams.

Urea (CON_2H_2).—Of the normal constituents of the urine

urea is much the most abundant and of the greatest physiological importance and clinical significance, being the chief representative of the katabolism of the nitrogenous tissues. The three chief end products of body katabolism are H_2O , CO_2 , and H_2N ; H_2O and CO_2 represent the breaking down of carbohydrates and fats; all three, and most characteristically H_2N , represent the breaking down of the vital nitrogenous bodies. Urea may be regarded as a combination of CO_2 and H_2N ($CO_2 + 2H_2N = CON_2H_4 + H_2O$). CO_2 is excreted mainly by the lungs, H_2O by the lungs, skin, and kidneys, H_2N mainly in the form of urea by the kidneys.

Urea is freely soluble and always appears in the urine in solution. In concentrated solution it is precipitated by nitric acid in crystalline form as urea nitrate; in the nitric-acid contact test with concentrated urines (with a urea content of about 5 per cent or over) a crystalline layer of urea nitrate frequently develops at the junction of the two fluids.

Under the influence of certain bacteria, which frequently find access to urine both before and after its passage, urea is converted to ammonium carbonate ($CON_2H_4 + (H_2O)_2 = (NH_4)_2CO_3$). This is the ammoniacal fermentation of the urine, which may take place within the bladder in cystitic and paralytic conditions, or after passage and standing of the urine.

Urea is decomposed into carbon dioxide and nitrogen by sodium hypobromite or hypochlorite, which affords means for its estimation. It is similarly decomposed by nitrous acid, so that frequently in the contact test with nitric acid bubbles collect in the fluids from decomposed urea, or on adding impure nitric acid to urine a slight amount of effervescence occurs.

The urea excreted originates partly from ingested proteid food, and to a greater extent from the katabolic metamorphosis of the nitrogenous body tissues. That derived from food being taken into account, its amount affords an index of the condition of the nitrogenous metabolism of the body. Since the liver is actively concerned in the elaboration of urea, and the kidneys in its excretion, the amount of urea excreted is also significant as to the efficiency of the liver and kidneys in these respects.

The daily amount of urea ordinarily excreted in the urine ranges from about 25 to 35 grams, or from 1.5 to 2.5 per cent of the urine. It is increased during the augmented tissue change caused by bodily activity, febrile diseases, and other conditions,

in diabetes mellitus, and with a meat diet. It is decreased in cachexias and conditions of lowered vitality, in uræmia, in kidney diseases with impaired renal excretory power, and in affections of the liver (notably cirrhosis, yellow fever, and acute yellow atrophy) in which the urea-forming functions of this organ are impaired. It is greater in males than females and relatively greater in children.

Xanthin Bases.—The xanthin or alloxur bases are a group of closely related basic substances that enter into the formation of the nucleins or essential chemical constituents of the nuclei of the body cells, and appear during the katabolic breaking down of the latter. They occur in animal and vegetable tissues, and in the urine are derived both from ingested food and nuclear katabolism. Those that have been found in the urine are xanthin ($C_5H_4N_4O_6$), hypoxanthin, heteroxanthin, paraxanthin, guanin, adenin; they occur in the urine as such only in minute amount, the largest part of them being apparently converted by oxidation into uric acid and so excreted. Ordinarily the quantity of the xanthin bases is only about a tenth that of the uric acid. To a certain extent (but not always) these bases therefore have the same origin, significance, and fluctuations as uric acid.

These substances occur in the urine in solution; xanthin very rarely appears in the form of undissolved, colorless, fusiform crystals, or enters into the formation of vesical calculi. The quantity of the xanthin bases is so minute that satisfactory methods for their practical determination are not available; hence their clinical significance apart from that of uric acid is as yet unsettled.

Uric Acid ($C_5H_4N_4O_6$).—This substance together with the xanthin bases make up the class called the alloxur bodies. Considerable clinical importance is attached to uric acid, but the question of its relation to disease processes is at present in a very unsettled state. As to its origin uric acid is now generally considered to be an oxidation product of the xanthin bases, and the chief form in which these bases are excreted; it is therefore the chief representative of the products produced by the katabolic breaking down of the cell nuclei of the body (of leucocytes especially). In man and mammals, then, uric acid is chiefly derived from certain foods and from nuclear destruction. To a certain extent uric acid can also be generated synthetically in the organ-

ism, and it is also partially capable of further oxidization into urea; these obscure and uncertain factors complicate the question of uric-acid formation and significance. In birds uric acid plays a larger part, taking the place of urea as the principal end product of nitrogenous katabolism.

The amount of uric acid excreted daily in the urine ranges from .4 to .8 gram, bearing a normal ratio to the amount of urea of between 1 to 40 and 1 to 60. It is increased after the ingestion of food rich in cell nuclei, as liver, kidneys, etc. (rather than muscle); in conditions attended with leucocytosis and hence with increased disintegration of nuclei, as in leukæmia (in which there may be a great increase of uric acid, up to 5 grams or more in twenty-four hours), in pneumonia (especially at the crisis), at the subsidence of digestion leucocytosis, etc.; in febrile conditions; in the uric-acid diathesis; and in other conditions.

It is diminished in anæmic and leucopenic conditions, chronic nephritis, and other cases. The relation of uric acid to gout and lithæmia is very obscure at present; uric acid itself does not seem to be responsible for the toxæmic manifestations in these diseases; yet it appears to fluctuate in amount parallel with the symptoms so as to serve as a clinical index of the toxic agencies, though itself innocuous.

Uric acid is very feebly soluble in water, requiring 17,000 parts of water at 20° C. or 1,900 parts of hot water for solution. It occurs in the urine both in uncombined form and in combination with the alkaline bases as sodium, potassium, and ammonium urates; each of these forms again may be in solution or in undissolved crystalline or amorphous state. As the urates are more soluble than uncombined uric acid, a larger amount of the acid can be held in solution in combined than in uncombined form.

In strongly acid urine, or after the addition of strong acids (hydrochloric, acetic), uric acid is freed from the urates, and if in excess of the soluble amount, especially in the cold, is precipitated in crystalline form. In alkaline or feebly acid urine, or after the addition of alkalies, the uric acid forms urates that are more soluble.

The crystalline forms of uric acid that appear in the urine (Fig. 21), while exhibiting considerable diversity of form, are usually very characteristic and easily recognizable. The urine in which the crystals appear is commonly very distinctive, being

concentrated, clear, of deep amber color, high specific gravity, strongly acid, and with strong urinous odor. The larger crystals are visible to the naked eye as minute reddish particles, resembling grains of red pepper or brick-dust on the bottom, or adhering to the sides of the receptacle containing the fluid. Under the microscope the crystals present varying forms; they are mostly of a more or less bright amber color, but are sometimes only faintly tinged or quite colorless; they vary in size from 10 to 20 micromillimetres in length up to particles distinguishable macroscopically. In form the crystals are frequently flat, with two parallel plane surfaces bounded by a double convex or lozenge-shaped contour; many deviations from this type appear, some slender and fusiform, some with curved outlines, some rhombic, some crossed, some short and thick, appearing rectangular, barrel-shaped, or fusiform, according to the point of



FIG. 21.—Uric-acid Crystals of Various Shapes.

view. Often the crystals are united in radiating clusters. Another type of uric-acid crystals is slender and prismatic. The crystals are very polymorphous, but usually characteristic enough to be easily recognized.

The presence of undissolved uric-acid crystals in urinary sediment does not necessarily indicate an excess of uric acid in the urine; their precipitation is caused essentially by concentration and strong acidity of the urine, with cold as an auxiliary, and may occur when uric acid is normal as well as when it is increased.

Urates.—Uric acid occurs in the urine chiefly in the form of sodium, potassium, ammonium, calcium, and magnesium urates, except when from high acidity it is forced from these combinations. The urates are considerably more soluble than uric acid, and more soluble in warm than in cold fluids. Many urines which at the body temperature or higher are perfectly clear and bright amber in color, when cold become turbid and dirty yellow

and precipitate their urates in the form of an abundant fine white or pinkish sediment (brick-dust or lateritious sediment), which is quite distinctive in appearance. The urates (except of ammonium) are freely soluble in alkaline fluids, and urine turbid with urates becomes clear on the addition of alkali. The mixed sodium and potassium urates are the commonest forms, calcium and magnesium urates being exceptional and minute in amount; ammonium urate is formed in connection with ammoniacal fermentation of the urine.

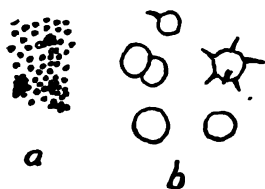


FIG. 22.—Urates. *a*, Amorphous granular urates; *b*, spherules of ammonium urate.

Microscopically the mixed urates (Fig. 22) usually appear as minute granules, sometimes irregular and amorphous, sometimes spherical, very minute, and faintly amber. Very

rarely sodium urate appears in crystalline form, as spherules, radiating prismatic crystals, or dumb-bell forms.

Ammonium urate is occasionally formed and precipitated during ammoniacal fermentation. It is the only urate that remains undissolved in alkaline urine, the other urates being precipitated only in acid media. Ammonium urate appears in the form of rather large amber-colored spherules, some with projecting spicules (Fig. 22).

Allantoin is a substance allied to uric acid that occurs in minute traces in normal urine, especially after meat diet; it is more abundant in the urine of newborn infants, and after the administration of tannic acid. It has no clinical importance.

Hippuric acid ($C_9H_7NO_3$) is a constant ingredient of normal human urine, in small quantity, about .5 to 1 gram daily. It originates to a small extent from metabolic products, to a larger extent from ingested food or drugs. It is much increased in amount after the ingestion of benzoic acid and some other substances, which are excreted as hippuric acid. It is also greater in amount on a vegetable diet, especially after the ingestion of fruits containing benzoic acid; it is very abundant in the urine of herbivora. It is still excreted, however, on a meat diet. It is increased in diabetes mellitus, chorea, acute fevers, and other conditions; decreased in chronic nephritis.

Hippuric acid is somewhat more soluble than uric acid; but when in excess, as after taking benzoic acid, its crystals may be separated in the form of long four-sided prisms with bevelled ends, or slender acicular crystals. The crystals are either separate or grouped together in radiating clusters. It is more soluble in alkaline than acid media, and occurs combined with the alkaline and earthy bases except when set free by strongly acid reaction.

Benzoic acid is occasionally present in the urine along with or in place of hippuric acid, as in diabetes and decomposing urine.

Creatin and Creatinin.—Creatin is a nitrogenous body ($C_4H_7N_3O_2$) occurring in muscle tissue; creatinin is closely related to it, containing one molecule of H_2O less. Both substances, especially creatinin, occur dissolved in the urine in small amount (.5 to .9 gram daily), derived partly from the body muscle tissue, partly from muscle ingested as food. The amount excreted increases on lean meat diet and in acute and certain other diseases; and is diminished in certain cases. It is not customary to consider these substances for clinical purposes.

Leucin and tyrosin occur in urine in appreciable amount only in abnormal conditions. They are closely related nitrogenous bodies, and usually occur together in the urine. They originate from abnormal proteid decomposition, or when the process of urea-formation is interfered with, as in hepatic disease. They thus appear in the urine in acute yellow atrophy of the liver, in large amount; in smaller amount in extensive suppuration or gangrene, hepatic carcinoma, cirrhosis, and other affections, leukæmia, typhoid fever, etc. When in large amount they may replace or supplement urea. They remain in solution, except that when they pass the point of saturation they appear in undissolved form; leucin as brownish spherules with fine concentric and radiating markings; tyrosin as fine acicular crystals arranged in sheaves, bundles, or radiating rosettes.

Ammonia, H_3N , may be regarded as the simplest ultimate end product of nitrogenous katabolism, and as such is excreted in large amount, almost entirely in a dehydrated combination with carbon dioxide as urea. In ammoniacal fermentation ammonium carbonates are generated in large amount. Aside from urea, ammonia compounds, similar to the corresponding sodium and potassium salts, are normally excreted in minute amount; the nitrogen of ammonia is normally about 2 or 3 per cent of the total nitrogen. In certain acid toxæmias in which there is an excessive formation of acids in the system from perverted metabolism (as in diabetic coma), or where there is excessive ingestion of acids, ammonia is excreted in the urine in excessive amount, in combination with these acids; in such cases the ammonia nitrogen is about 18 to 25 per cent of the total nitrogen, and an approach to this ratio is a danger signal. The rationale of this is that the sodium or potassium bases are not sufficient in amount to neutralize all the acid, and the ammonia which would otherwise go to form urea combines with the excess of acid and is so excreted. Quantitative estimation of ammonium compounds in the urine therefore furnishes an index of katabolic acid formation.

The ammonium salts of the urine form "volatile" alkali, in contradistinction to the "fixed" alkalies, sodium and potassium.

Total Nitrogen.—The substances thus far considered comprehend the chief katabolic nitrogenous products, and sometimes their total nitrogen content is measured as an index of the body nitrogen katabolism more complete and representative than the amount of urea alone affords. Ordinarily the total nitrogen excreted in the urine daily ranges from about 15 to 21 grams, of which about four-fifths is furnished by urea.

Sulphur Compounds.—The sulphur excreted in the urine, like urea, chiefly results from the decomposition of albuminous material, either that ingested with the food, or that broken down in katabolic processes. The sulphur is excreted chiefly in the form of sulphates; also in unoxidized combinations, in traces in normal urine, in larger amount (cystin, hydrogen sulphide) in abnormal conditions.

Sulphates.—These are normally excreted in quantities of 1.5 to 3 grams daily. Their quantity in general fluctuates parallel with that of urea. They are increased by meat diet, the ingestion of sulphuric acid or sulphates, exercise, in acute fevers, especially meningitis and rheumatism, in leukæmia, and other affections. The sulphates of the urine are of two kinds, mineral sulphates and the conjugate or ethereal sulphates.

Mineral Sulphates.—These ordinarily constitute about nine-tenths of the total urinary sulphates. They consist chiefly of sodium sulphate, with a small proportion of potassium and ammonium and perhaps calcium and magnesium sulphates. These salts, excepting calcium sulphate, occur in the urine only in solution. Calcium sulphate rarely appears in urinary sediments, in the form of slender prismatic or acicular crystals, some in radiating clusters, or in the form of amorphous granules or small dumb-bell shapes.

Conjugate or Ethereal Sulphates.—These are formed by the combination of potassium or sodium sulphate with indol, phenol, and related substances generated in the course of putrefactive decomposition of proteids. They are normally formed in the intestine from disintegration of proteid food material; and at times originate abnormally in putrid, suppurative, or necrotic processes, or abscesses. From the place of origin they are absorbed into the circulation and thence excreted by the kidney,

constituting normally about one-tenth of the excreted sulphates. These substances therefore represent albuminous putrefaction, and are of diagnostic significance in that respect. They are diminished or absent with a decrease or cessation of intestinal decomposition. They are increased, or their ratio to the mineral sulphates is increased, in conditions of increased gastric or intestinal decomposition, as in gastric carcinoma, gastritis, hypochlorhydria, gastric and intestinal stagnation, intestinal obstruction, peritonitis; also in putrid, suppurative, or necrotic conditions outside the alimentary tract, as in cystitis, empyema, etc.; and from the use of certain drugs.

The conjugate sulphates consist chiefly of indoxyl-potassium sulphate, with ordinarily a less amount of phenol-potassium sulphate, and traces of the potassium sulphates of cresol, catechol, and skatoxyl. These sulphates as such occur in the urine in solution.

Indoxyl-potassium sulphate, or **indican**, is the most abundant and most conspicuous of the conjugate sulphates, and ordinarily representative of them all, so that increase of indican has the same significance as that of the total ethereal sulphates. By decomposition with hydrochloric acid or otherwise, along with oxidation, as by the simultaneous or subsequent action of nitric acid or other oxidizing agents, indican is converted into a red or a blue pigment (indigo-red and indigo-blue), according to the amount present and stage of oxidation. Rarely this takes place spontaneously, or after urine has stood for a time, giving rise to blue or red urines. In decomposing urine indigo-blue is sometimes deposited in the form of undissolved amorphous blue granules, rarely in the form of delicate blue crystals; and a very few instances are reported of indigo calculi being formed in the kidney.

Unoxidized Sulphur Compounds.—Normally only traces of sulphur compounds other than sulphates occur in the urine, such as sulphocyanides derived from absorbed saliva, other substances perhaps of katabolic origin and related to cystin, and possibly derivatives of taurocholic acid from absorbed bile. The latter is present in choluria. Two bodies rarely present in abnormal conditions, cystin and hydrogen sulphide, are of some importance.

Cystin ($C_4H_8NSO_2$) rarely occurs in the urine. Its formation

is not well understood, but seems to depend on metabolic anomalies or hepatic disorder, arising either from proteid katabolism or from taurin in the liver. Cystinuria has little or no clinical significance; it may continue for years without material impairment of health, and is at times a hereditary anomaly.

Cystin is present in the urine both in solution and as an undissolved crystalline sediment, and also sometimes forms calculi. The crystals are hexagonal plates, occurring separately or in superimposed forms; they are soluble in the caustic alkalis and in oxalic and strong mineral acids; insoluble in ammonium-carbonate solution, and hence precipitated during ammoniacal fermentation. When occurring in solution only, without crystals to direct attention to it, its presence may not be suspected unless hydrogen sulphide arising from its decomposition is detected. To a certain degree cystinuria is apparently associated with the formation and excretion of certain toxic diamines.

Hydrogen sulphide, H_2S , is rarely encountered in the urine (hydrothionuria). Sometimes it is generated subsequent to its passage by decomposition processes or H_2S -forming bacteria acting on the sulphur-containing substances of the urine. It is especially apt to appear in urine containing cystin. At other times it is formed within the body economy, and then absorbed or excreted into the urine, as in putrefactive processes of the intestine (frequently associated with excess of indican), and in foul abscesses in which H_2S is generated. H_2S injected into the rectum may appear in the urine.

Phosphates.—The compounds of phosphoric acid excreted in the urine are derived from the surplus phosphates of the ingested food and from the waste products of the katabolism of the body tissues and substances containing phosphorus. As urea and its congeners represent nitrogenous katabolism, so the urinary phosphates, aside from those of the food, represent and indicate phosphoric katabolism. The principal body substances containing and yielding phosphorus are the nucleins, lecithin, and phosphates. A portion of the excreted phosphorus is derived from nuclear disintegration (nucleins), and this portion should bear a definite ratio to the uric acid and xanthin bases derived from the same source. Another portion is yielded by the breaking down of tissues containing lecithin and phosphates, especially nervous tissue, bone, blood corpuscles, and muscle; this portion

is independent of the excretion of the alloxur bodies. The actual excretion of the phosphates is dependent on the functional power of the kidneys in the same manner as that of urea.

The normal amount of phosphoric acid excreted daily is about 2.5 to 3.5 grams, measured as P_2O_5 . It varies with the amount of phosphates introduced with the food, being greater on animal than vegetable diet. Its fluctuations in disease are not completely made out. The excreted phosphates should be increased by increased katabolism of nuclei, nervous tissue, bone, blood corpuscles, etc.; or when (as in rickets) the ingested phosphates normally used up in the nutrition of the tissues (as the bones) are not utilized for this purpose, but instead are excreted unused.

They should be diminished in decreased phosphoric katabolism, or when there is an increased consumption of the ingested phosphates for tissue formation (as in pregnancy or rapidly developed leucocytosis), or when from impaired functional power of the kidneys or other causes the phosphates present in the blood are retained and not excreted in usual amount. Study of the ratio of the total amount of P_2O_5 to the total nitrogen excreted in the urine (normally 1 to 5 or 7) or of its ratio to the alloxur bodies, may reveal information significant as to the seat of the disturbance, whether due to abnormal metabolism of cell nuclei or of other structures.

Clinically, the total excretion of phosphates is decreased in most acute diseases (from excretory failure), anæmias, pregnancy, some nervous diseases, nephritis, some cases of hepatic disease (cirrhosis, acute yellow atrophy), and other conditions. The total excretion is increased in some bone diseases, in starvation, some nervous diseases, during sudden hæmocytolysis (as after rapid destruction of many red corpuscles or the subsidence of leucocytosis in convalescence from acute conditions), and in "phosphatic diabetes."

The combinations in which phosphoric acid appears in the urine are glycono-phosphoric acid (in minute traces), alkaline phosphates, earthy phosphates, and ammonio-magnesium phosphate.

Alkaline Phosphates.—These consist chiefly of sodium phosphates, with a small amount of potassium phosphates. Phosphoric acid is tribasic, and forms three series of salts. In the case of sodium, for instance, these are neutral sodium phosphate

(Na_2PO_4), which is alkaline in reaction, and monacid (Na_2HPO_4) and diacid (NaH_2PO_4) sodium phosphate, which are of acid reaction. All three of these may be present in the urine, but normally the acid phosphates preponderate, and to these the urine chiefly owes its acidity. When from any cause the neutral sodium phosphate increases in relative amount, the urine becomes proportionately less acid, neutral, or alkaline.

The sodium and potassium phosphates of the urine are proximately derived from those of the blood. Why these phosphates, which in the blood are alkaline, should pass into the urine in acid form is not altogether clear. These phosphates are freely soluble in aqueous fluids of any reaction, and occur in the urine only in solution. They are normally excreted to the amount of 2 to 4 grams daily.

Earthy Phosphates.—These are the phosphates of calcium and magnesium: $\text{Ca}_3(\text{PO}_4)_2$, normal or tricalcic phosphate, CaHPO_4 , the monacid phosphate, $\text{CaH}_2(\text{PO}_4)_2$, the diacid phosphate, $\text{Mg}_3(\text{PO}_4)_2$, MgHPO_4 , and $\text{MgH}_2(\text{PO}_4)_2$. The amount of these daily excreted is normally 1 to 1.5 gram, the amount of magnesium phosphates being about double that of the calcium phosphates. The earthy phosphates are only sparingly soluble in pure water, quite soluble in acid fluids, even if only slightly acid, insoluble in alkaline media. In urine they are held in solution by NaH_2PO_4 , CO_2 , and NaCl ; in urine that is alkaline or made alkaline they are thrown down as a white precipitate. The acid salts are more soluble than the normal earthy phosphates; and the three series of phosphates are readily transformed from one to another by changes in the surrounding conditions.

Under certain conditions the earthy phosphates are precipitated from urine by heat. In many specimens of urine a white cloudiness or precipitate appears on being heated in a test tube, which clears up when a drop of acid is added—a reaction which distinguishes phosphates from an albuminous precipitate. If the urine be cooled after the cloudiness has been developed by boiling, the precipitate totally or partially redissolves, and the fluid clears either entirely or in part. The precipitate formed by the heat consists of phosphates of calcium and magnesium. The precipitation is mainly due either (*a*) to the chemical nature of the phosphates being altered by heat, less soluble salts being formed, while the process is reversed on cooling and the salts

redissolve; or (b) to their being less soluble in hot than in cold media and being therefore thrown down by heat, redissolving on cooling. Another cause, which is perhaps especially manifest in those cases in which the turbidity persists after cooling, is the expulsion of CO_2 and consequent loss of acidity caused by boiling, which diminishes the solvent power of the urine over the earthy phosphates. The precipitation by heat will occur whenever the earthy phosphates are in solution in such degree of concentration that when heated the point of saturation is passed. The degree of concentration depends partly on the amount of earthy phosphates, partly on the acidity of the urine, and the concentration increases as the acidity diminishes. The precipitation by heat does not in itself necessarily indicate that the earthy phosphates are absolutely increased, and, in fact, is ordinarily due to a low degree of urinary acidity without any increase of phosphates.

The earthy phosphates may occur in the urine either in solution (in acid urine) or (in weakly acid or alkaline urines) undissolved. In the latter case they usually appear microscopically in the form of masses of fine, amorphous, colorless granules, which dissolve on the addition of acid. Calcium phosphate (Fig. 23) appears rarely in the form of slender prismatic or broader wedge-shaped crystals, usually in radiating masses. Magnesium phosphate also rarely occurs as short crystals.

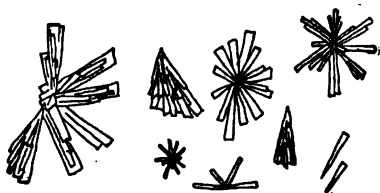


FIG. 23.—Calcium-phosphate Crystals.

In disease conditions the alkaline and earthy phosphates may fluctuate *pari passu*, or either may be increased or decreased independently of the other, according to the source of the phosphates at fault.

Ammonio-magnesium phosphate (triple phosphate, MgNH_4PO_4) is formed and precipitated whenever any dissolved phosphates, ammonium compounds, and magnesium salts are commingled in an alkaline medium. It is not excreted into the urine as such, but is formed in abundance in the process of ammoniacal fermentation of the urea, and is one of the characteristic features of that process. It is not soluble in alkaline fluids, and in am-

moniacal urine appears (mixed with calcium phosphate) as an abundant undissolved, white, powdery sediment, which microscopically is in the form of both amorphous granules and crystals. The crystals of triple phosphate are very distinctive, being

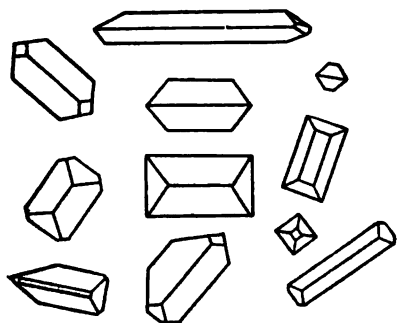


FIG. 24. Ammonio-magnesian Phosphate Crystals.

clear, colorless, triangular prisms with bevelled ends, mostly of relatively large size (Fig. 24); these are the forms found in the urine, and are produced when the triple phosphate or the crystals are formed very slowly. If the phosphate is suddenly precipitated, as by mixture of the necessary solutions in a test tube, delicate feathery and snowflake-like crystals

are produced; these after a time gradually change into the prismatic crystals.

Carbon dioxide and carbonates are ordinarily present in urine, originating from katabolism, from the food, and from ammoniacal transformation of urea. Carbon dioxide is one of the chief katabolic end-products, being excreted chiefly by the lungs, but to a small extent by the kidneys also. The food and other ingesta are an important source, salts of organic acids in particular being excreted as carbonates. In ammoniacal fermentation, large quantities of ammonium carbonate are formed from urea. Both free carbon dioxide and carbonates are present in solution in the blood plasma, and each is capable of excretion as such into the urine; but the free gas is best adapted to elimination by the lungs, the carbonates by the kidneys.

Carbon dioxide is practically constantly present in urine in free form, and sometimes also in alkaline urines in combination as carbonates. The free CO_2 of the urine contributes materially to its acidity, and aids considerably in keeping the earthy phosphates in solution. As it is a volatile acid principle, urine is diminished in acidity after its expulsion by boiling, agitation, or otherwise; and if the acidity of the urine be entirely due to CO_2 , litmus paper reddened by it turns blue again on drying. The free CO_2 originates partly from direct passage of the gas from

the blood into the urine, partly from decomposition of carbonates excreted into acid urine.

Carbonates and bicarbonates are frequently present in alkaline, neutral, or feebly acid urines, but cannot exist as such in acid urines, which immediately decompose them and set the CO_2 free. The carbonates thus present are ordinarily those of sodium, potassium, and ammonium, the latter especially in ammoniacal fermentation; calcium and magnesium carbonates may also be present. Calcium carbonate sometimes appears undissolved in the sediment of alkaline urine, in the form of small granules, spherules, or dumb-bell forms which effervesce on adding acid; rarely it forms vesical calculi. The carbonates increase the alkalinity of urine. Ammonium carbonate is "volatile alkali," and is dissipated by heat; litmus paper turned blue by dissolved ammonium carbonate reddens again on drying. Sodium and potassium carbonates are "fixed alkalies," and not affected by heat.

The amount of free CO_2 present in acid urine under ordinary circumstances ranges from about 2 to 12 per cent by volume (.004 to .025 per cent by weight). Free or combined, the amount is increased in neutral and alkaline urines, after vegetable diet or the ingestion of alkaline carbonates or salts of organic acids, after drinking carbonated waters, and during conditions of heightened metabolism, as exercise or fever. After ingestion of large amounts of calcium hydrate, calcium carbamate appears in the urine, and decomposes, yielding CO_2 , CaCO_3 , and H_2N .

Chlorides, next to urea, form the most abundant single constituent of normal urine, 10 to 16 grams being excreted ordinarily each twenty-four hours. The chlorides excreted consist chiefly of sodium chloride, with a small proportion of potassium, ammonium, calcium, and magnesium chlorides. They are derived from the surplus, over body needs, of chlorides introduced with the food, and are not products of body katabolism.

The chlorides are excreted in diminished amount, even to practical absence, in most acute febrile diseases, the diminution being proportionate to the severity of the disease; and they increase again with the subsidence of the disease; their decrease and increase in such cases are therefore of unfavorable and favorable import respectively and correspondingly. The chlorides in the urine are also diminished in connection with excessive secretion of gastric juice or with the development of copious se-

rous exudations or transudations (the chlorides in such conditions being otherwise disposed of) and in various other affections.

They are increased after the use of certain drugs, after a period of retention of the chlorides (as in convalescence from acute fevers), during the absorption of exudates and transudates, and in other conditions. They fluctuate also to a certain extent with the amount of urine excreted, being increased in daily quantity in polyuric conditions, and vice versa.

Calcium oxalate, in small amount, frequently occurs in both acid and alkaline urines in the form of minute undissolved crystals. It originates both from food (especially acid fruits, etc.)

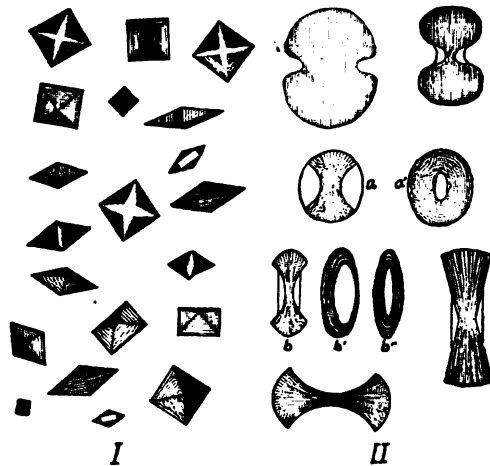


FIG. 25.—Calcium-oxalate Crystals. I, Octahedral forms. II, "Dumb-bell" forms. *a*, *a'*, One crystal from two points of view; *b*, *b'*, *b''*, one crystal from three points of view.

and from metabolic processes in a manner not well understood. When constantly and abundantly present the crystals may indicate some nutritive or nervous disturbance, though their significance is not well defined; often they appear in the urine of persons in good health without having any pathologic import at all.

The crystals appear in two forms (Fig. 25). By far the commonest form is that of minute, highly refractile, colorless octahedra, varying in size from about 3 to 15 micromillimetres. Viewed from an apex, they present a very characteristic "envelope shape," the form of a square crossed by diagonals. Some of the octahedra are regular; some, usually smaller in size, elon-

gated in lozenge form; some flattened. The crystals occur separate, attached together in masses or strings, or adhering to mucous threads, casts, etc.

The other forms of calcium-oxalate crystals are the so-called "dumb-bell forms," which are uncommon. They vary somewhat in form, sometimes being oblong, with an annular constriction in the middle (dumb-bell shape). Often they are flattened spherical or oval form, deeply biconcave from a depression at opposite sides; these forms have the dumb-bell appearance when viewed from one direction, and appear oval when viewed from another. They are sometimes minute, but are usually much larger than the octahedral forms, of duller lustre, and less refractile, and exhibit longitudinal lines arranged radially or concentrically, according to the point of view.

Pigments and Chromogens.—The substances to which urine owes its color, and those (the chromogens) which while themselves colorless under special circumstances or treatment develop coloring matter, comprise a group of diverse substances having little in common as to nature and significance except their color.

Present knowledge and nomenclature of the normal and some abnormal urinary pigments are very confused and imperfect. The pigments and chromogens of urine, normal and abnormal, are about as follows: urochrome, uroerythrin, indican and indigo, an unnamed substance in febrile urines, bilirubin, a slightly altered form of bilirubin, hæmoglobin and its derivatives, melanin, and adventitious coloring matters derived from ingesta.

Some of the pigments and chromogens exhibit noticeable reactions with nitric acid in the course of the tests for albumin, which direct attention to them. In the contact test, zones of color form at the junction of the fluids; or after heating the urine the addition of a drop or two of nitric acid causes color change.

Urochrome.—This is the chief or only pigment of normal urine, to which its yellow color is due. By some it is called urobilin, while others apply the term urobilin to an abnormal pigment. The term urochrome, here adopted, seems admirably descriptive and suitable as a designation for the normal urinary coloring matter. Its composition and origin are not definitely determined, and it probably has not yet been isolated in purity. In normal amount it probably gives little or no color change

with nitric acid. It appears to be proximately a derivative of bilirubin, and through that of hæmoglobin. It is hence decreased (the urine being paler) in anæmic conditions, where the amount of hæmoglobin set free by erythrocytolysis is diminished; and is increased in febrile conditions or during the absorption of hemorrhagic extravasations, where there is an increased breaking down of red blood corpuscles.

Uroerythrin is the rosy pigment which colors certain urinary sediments, especially undissolved urates. Urine containing it in excess may stain paper pink. Opinions differ as to whether it is present normally or abnormally, and as to the extent to which the high color of febrile and other concentrated urines is due to it.

Indican, already considered, is a normal chromogen from which indigo-red and indigo-blue may be derived. Rarely this takes place spontaneously, after standing, giving rise to red or blue urines. The pink color that appears in the nitric-acid tests with normal urine is probably due to indigo-red.

The high color of febrile and other concentrated urines may be partly due to an increased amount of urochrome or uroerythrin; but in addition to these an unnamed and unknown substance is perhaps present. Such urines in the nitric-acid contact test yield bright to dark red, purple, or dark color zones at the junction of the fluids, or the entire urine turns a similar color after being heated and treated with a few drops of nitric acid.

Bilirubin and an altered form of bilirubin not capable of being oxidized to biliverdin appear in the urine in icteric conditions and choluria, imparting a brown or deep amber color (see below).

Hæmoglobin and certain of its derivatives when present give the urine a color ranging from blood red to dark shades (see below).

Melanin.—In some (not all) cases of melanosis or melanotic tumors the black pigment melanin may appear in the urine, either in solution or in the form of undissolved black granules. Usually the urine is normal in color on being passed, but turns dark or black on standing, the substance actually excreted being a chromogen (melanogen).. In wasting diseases the urine sometimes contains melanin. A black pigment (probably different from melanin) is sometimes voided with the urine in repeated or

chronic malaria. Urines containing alkapton also turn dark on standing.

Various ingested foods and drugs yield substances that affect the color of the urine (page 164).

Fat is sometimes present in the urine in minute amount in the form of small globules discernible under the microscope; rarely the fat is very abundant (lipuria), so that it runs together in oily masses or rises to the surface of the urine as a layer of fluid oil, a cream-like layer, or (on cooling) a solidified or coagulum-like layer. Urine containing much fat, suspended in fine globules, is whitish, opaque, and milk-like. Very rarely fatty calculi form in the bladder.

In small amount fat may be present as droplets in or set free from epithelium cells that have undergone fatty degeneration in parenchymatous inflammations or fatty conditions of the kidneys; the fatty casts are of like origin. Fat has been observed in the urine, sometimes in large amounts, during the repair of fractures, fat embolism, heart disease, pancreatic disease, diabetes mellitus, phosphorus poisoning, abscesses and necrosis of adipose tissue (liberating fat in abundance), especially when discharging into the urinary passages, during pregnancy and the puerperium, following the ingestion of oils (as *oleum morrhue*), from the rupture and leakage of lymph vessels into the ureter or bladder, and in other conditions from causes imperfectly understood.

Fat is most often present and abundant in the urine in chyluria, a tropical affection mostly associated with filariasis and due to leakage of chyle into the urine. Chylous urine is white, opaque, and milk-like; it contains large amounts of fat in very fine granular subdivision, along with leucocytes, albumin, coagulable fibrin, red blood corpuscles, and sometimes larval filariæ.

In cases of lipuria not chylous the fat occurs in larger globules than in chylous urine.

Fatty acids (formic, acetic, butyric, propionic) occur in the urine normally in minute amount, increased at times in various conditions, as diabetic coma. They are derived from intestinal fermentations, and perhaps in some cases from perverted katabolism. In the course of cystitis in cases of glycosuria they may originate from fermentation of the glucose. **Earthy soaps** are perhaps at times present in the urine in the form of radiating acicular crystals. **Lactic acid** has been found in the urine after muscular exercise, in hepatic affections, diabetic coma, and other conditions. Other **organic**

acids, ferments (pepsin, diastase, rennet), and **hydrogen dioxide** have been demonstrated in urine in traces, but are without practical importance. **Iron** is normally present in minute amount, probably entering into the formation of some of the pigments. **Nitrates**, derived from ingesta, sometimes appear in the urine in small amount. Traces of **silicic acid** may be present. Small amounts of free **oxygen** and **nitrogen** are normally present, derived from the inspired gases taken into the blood. The other gases of the urine, CO_2 and H_2S , have been already considered.

A few cases have been reported in which a large amount of gas was present in the urine (pneumaturia), distended the bladder, or escaped from the urethra. Some cases are caused by the entrance of gases from the intestine through a vesico-enteric fistula. Others arise from the action of gas-forming bacteria on the urine in the bladder, being usually associated with cystitis. Many of these cases occur in glycosuric conditions, the sugar being fermented by microorganisms like yeasts and the colon bacillus, and yielding CO_2 , alcohol, and fatty acids. Some cases occur in connection with sugar-free urines, the substance yielding the gas not being known; the colon group, bacillus aerogenes capsulatus, and other bacteria are the active agents, and the gases produced are carbon dioxide, hydrogen, oxygen, nitrogen, and methane. Exceptionally the site of gas generation is the kidney or ureter.

Proteids.—The proteids that appear in the urine, chiefly in abnormal conditions, are nucleo-albumin, serum-albumin, globulin, albumose, fibrin, hæmoglobin.

Nucleo-albumin, or urinary mucin, is often present in normal urine in minute amount, at other times absent or so scanty as to be undemonstrable by delicate tests. It may be in solution, or it may in strongly acid urine be precipitated in the form of fine, colorless **mucous threads**, which play an important part in the formation of the light flocculent cloud or nubecula that often forms in urine on standing. If in excess mucin sometimes appears in the form of viscid, gelatinous, mucous masses, which settle to the bottom unmixed with the urine, or rarely may give the entire urine a gelatinous consistency. It is normally derived from the mucous lining of the urinary passages; but as this mucous membrane lacks mucous glands and goblet cells, its mucinogenous action ordinarily is very insignificant. In women it may be adventitiously introduced from the vagina. Mucin is increased in the urine by irritation or catarrhal involvement of the uriniferous tubules and urinary tract; also sometimes in acute nephritis, febrile and other albuminurias, choluria, leukæmia, etc. Its increase is chiefly due to pathologically increased mucinogenous action of the renal or urinary cells, or to disintegration of these cells; in some cases (as in choluria, leukæmia,

and perhaps other conditions of leucocytolysis) it is probably introduced from the blood.

Albumin (serum-albumin) is the most important urinary proteid. It is never normally present in urine, except possibly in traces so minute as to be demonstrable only by the most delicate tests, and even then with doubt. Its presence (at least in amount sufficient to respond to the ordinary tests) is always of serious pathological significance, and the test for albumin is one of the most important items in the examination of urine. Albumin may and most frequently does appear in the urine as a result of impaired functional action of the parenchymatous renal cells, and is thus indicative of acute or chronic nephritis, renal congestion, toxic injuries to the renal cells (as in febrile toxæmias, etc.), parenchymatous renal degeneration; albuminuria may also result from circulatory conditions (as increased renal blood pressure), alterations in the quality of the blood, and in other ways not well understood. Albumin may also enter the urine along with blood, lymph, or pus from lesions along the urinary passages subsequent to the excretion of the urine from the kidneys. The greatest amount of albumin usually occurs in the urine in acute and chronic parenchymatous nephritis; a less amount in other conditions. The amount of albumin excreted may range up to 10 or 15 grams per day.

Globulin (serum-globulin) usually appears in the urine whenever serum-albumin does, in large relative amount, sometimes indeed in greater quantity than the latter. Its origin and significance are about the same as those of serum-albumin.

Egg-albumin sometimes appears in the urine after free ingestion of eggs as food.

Albumoses may appear in the urine in a variety of conditions, as in association with other albumins in albuminurias, in suppurations, bone diseases, hepatic affections, and many others. The definite clinical significance of albumosuria is not determined.

Whether *peptone* ever appears in the urine is now in doubt, the reactions formerly taken as showing the presence of peptones now being regarded as indicative of albumoses and not of peptone.

Fibrin appears in the urine in hæmaturia, chyluria, lymphuria, or fibrinous inflammation of the urinary tract. The passage

of blood, chyle, or lymph into the urine necessarily introduces fibrin, with other substances. The fibrin may be in solution in the urine, in coagula of various size, or in microscopic flakes; sometimes the entire urine coagulates into a gelatinous mass.

Other proteid substances, varying from the ordinary proteids in some of their reactions, are rarely found in the urine, as histon.

Carbohydrates.—This urinary group includes one substance of prime importance, glucose, along with a number of rare or less important related substances, namely, levulose, inosit, sucrose, lactose, maltose, pentoses, glycogen, dextrin, animal gum.

Glucose (dextrose, grape sugar), $C_6H_{12}O_6$, is usually practically absent from normal urine, though traces of it or some other reducing agent may at times be demonstrable in concentrated urines. It may appear temporarily in many diverse conditions, as febrile affections, meningitis, alimentary disturbances, hepatic disease, excessive ingestion of starchy or saccharine foods, or after the use of some drugs, as phloridzin; such temporary occurrence has no serious significance. Its persistent presence in large quantities in the urine is the cardinal and pathognomonic symptom of diabetes mellitus, of pancreatic, hepatic, nervous, or any other form, and indicates severe metabolic disturbance.

According to its abundance glucose causes a marked increase in the specific gravity of the urine, frequently up to 1.040 or more; it is sometimes, however, plentiful even with low specific gravities, down to 1.012. The amount eliminated in twenty-four hours ranges from zero up to 500 grams. With the glucose other allied carbohydrates may at times appear.

Glucose when boiled with caustic alkali with access to the air is oxidized and turns dark-colored. It reduces the oxides of copper and bismuth. With phenylhydrazin it forms crystals of phenylglucosazon. It rotates polarized light to the right. Under the action of certain micro-organisms, as yeast and the colon bacillus, glucose undergoes fermentation, breaking up into carbon dioxide and alcohol ($C_6H_{12}O_6 = 2C_2H_5O + 2CO_2$); fatty acids and other substances may also be formed in the process.

At times in cases of glycosuria when the bladder becomes infected with the fermenting organisms, the glucose undergoes fermentation in the bladder, with the formation of carbon dioxide, alcohol, and sometimes fatty acids, which are voided with

the urine. Thus, in an intercurrent cystitis in the course of diabetes mellitus, the amount of glucose in the urine may diminish, abundant gas, with alcohol, may be present, and the urine may be strongly acid. Such a decrease must not be mistaken for a real decrease in the renal excretion of glucose.

Levulose, $C_6H_{12}O_6$, sometimes appears in the urine in diabetes mellitus, usually together with glucose. Its chemical reactions are similar to those of glucose, except that it rotates polarized light to the left.

Inosit, $C_6H_{12}O_6 \cdot 2H_2O$, is occasionally present in the urine in diabetes mellitus, diabetes insipidus, and chronic nephritis, and at times in other conditions, and even in traces in normal urine. It is quite different from glucose in its reactions, having no action on polarized light, not undergoing alcoholic fermentation with yeast, not giving the phenylhydrazin reaction, and not typically reducing copper.

Sucrose (saccharose or cane sugar), $C_{12}H_{22}O_{11}$, may appear in the urine in traces after the free ingestion of cane sugar. It is dextro-rotatory, and does not reduce copper, or ferment with yeast. By prolonged boiling with water (especially if acidulated) sucrose is inverted, or converted into a mixture of glucose and levulose, with a predominating lævo-rotatory power.

Lactose (or milk sugar), $C_{12}H_{22}O_{11} \cdot H_2O$, is frequently present in small amount (exceptionally in large amount, constituting a sort of diabetes) at the end of pregnancy and during lactation, especially with amply developed breasts or when the milk secreted is not freely evacuated, as just after labor, during mastitis, and at the weaning period. Lactose reduces copper, is dextro-rotatory, gives (in dilute solution) a negative phenylhydrazin reaction; it does not undergo alcoholic and CO_2 fermentation with yeast, but is more subject to lactic-acid fermentation. It may be converted into glucose and galactose.

Maltose, $C_{12}H_{22}O_{11}$, has been observed in urine along with glucose. Its reactions are similar to those of glucose. Maltose is the first sugar formed in the diastatic digestion of starch; it is closely related to glucose, into which it is converted by further action of diastase or other treatment.

Pentoses.—This is a group of sugars based on the formula $C_5H_{10}O_5$, some of which (arabinose, rhamnose, xylose) may appear in the urine after the ingestion of certain food (pears and other fruit, beer, etc.), in diabetes, and in other conditions.

Glycogen, $nC_6H_{10}O_5$, has been observed in the urine, in diabetes and other conditions.

Dextrin, $nC_6H_{10}O_5$, has also been observed in the urine in diabetes mellitus.

Animal gum is said to be present even in normal urine in minute amount.

Glycuronic acid, $C_6H_{10}O_7$, is related to the carbohydrates, and may occur in the urine in combination with alkaline or with ethereal bases (indoxyl, phenol, etc.). Glycuronates sometimes appear in normal urine in traces and in increased amount after the ingestion of turpentine, camphor, chloral, chloroform, morphia, phenol, and other drugs. Of their quantitative variations in disease conditions practically nothing is known. Glycuronic acid in the

urine probably has little clinical significance, but it is of practical importance in that it reduces copper salts and is hence apt to be erroneously taken for glucose.

Alkapton.—Cases are rarely met in which the urine contains a reducing substance of obscure origin and composition, but different from the sugars, glycuronic acid, or certain reducing ingesta. Various names have been given this substance, as pyrocatechin, urrhodinic acid, oxyphenic acid, glycosuric acid, homogentisinic acid, etc.; but until its definite nature is ascertained the term "alkapton," employed in the first case studied, may be conveniently retained for the substance or substances responsible for the reactions presented. Urines containing alkapton may be of normal color on being passed; but, especially when alkaline or made alkaline by fermentation or the addition of caustic alkali, they turn dark from the surface of the urine downward, by a process of oxidation; they reduce salts of copper, but not of bismuth, do not ferment with yeast, do not affect polarized light, and give no phenylhydrazin reaction. Alkaptonuria has been observed both in healthy and diseased persons, but is probably in itself an anomaly devoid of pathologic significance. It may persist for years, has been discovered in both children and adults, and may appear in several individuals of the same family. Practically it is important not to mistake it for glycosuria.

COMPARATIVE TABLE OF REACTIONS OF CARBOHYDRATES AND ALLIED BODIES.

Substances.	Reduces copper salts?	Undergoes alcoholic fermentation with yeast?	Rotatory action on polarized light.	Gives phenylhydrazin reaction?	Melting-point of osazon crystals in phenylhydrazin test.	Reaction with KOH in contact with air.
Glucose	Yes	Yes....	Right, 56°	Yes	204° C ...	Darkens when boiled.
Levulose	Yes	Yes....	Left, 106° at 14° C.	Yes	150°	Darkens when boiled.
Inosit	Not typically...	No	None.....	No.		
Sucrose	No	No	Right, 74°	No		None.
Lactose	Yes	No	Right, 59°	Not in dilute solution.		None.
Maltose	Yes	Yes ...	Right, 150°	Yes	190°	Slightly discolored by boiling.
Pentoses	Yes	No	Some to right, others inactive	Some, yes.		
Glycogen	Not typically...		Right, 211°	No.		
Dextrin	No		Right, 138°	No		None.
Animal gum ...	No.					
Glycuronic acid.	Yes	No	Right	Yes.		
Glycuronates ...	Yes	No	Left	No.		
Alkapton	Yes	No	None.....	No		Darkens at ordinary temperature.

Acetone, Diacetic Acid, β -Oxybutyric Acid.—These three substances are closely related chemically and pathologically. They occur separately or associated together, especially in diabetic coma and other manifestations of the "acid intoxication"; they

also at times appear in other conditions. Their precise relations to one another and to the disease process are not as yet determined. Their presence in abundance is usually accompanied by an increase of ammonia, which forms ammonium salts with these acids and is abstracted from that which would otherwise form urea.

Acetone seems to be a product of disintegration of fat or proteid, either from food or body tissue, and in minute amount may appear in normal urine. It may be increased on a fat or meat diet, with insufficient carbohydrate food, during starvation, high fevers, nervous and mental affections, carcinoma, alimentary disturbances; and pre-eminently in diabetes. Acetone imparts a fruity odor to the urine, is volatile, and expelled by boiling.

Diacetic acid and β -oxybutyric acid are never present in normal urine; their presence and that of acetone in excess are of serious import. Diacetic acid yields similar reactions to those of acetone, but it is not volatile; under certain conditions it breaks up and yields acetone, alcohol, and carbon dioxide. Beta-oxybutyric acid is prominently characterized by its lævo-rotatory power.

Alcohol may rarely be found in the urine, in traces after free ingestion of alcohol, in connection with diacetic acid as one of its decomposition products, and in cases of glycosuria when the glucose has undergone alcoholic fermentation in the bladder.

Blood (hæmaturia).—Blood, as such, enters the urine by hemorrhagic extravasation in some part of the urinary tract. All the elements of the blood are thus introduced into the urine, plasma, corpuscles, and pigment. Hæmaturia is manifested by the concurrent presence of proteids (albumin, globulin, sometimes clots of fibrin), red corpuscles, leucocytes, and hæmoglobin or its derivatives.

The characteristics and appearance of urine containing blood depend upon the amount of blood present, its source, the length of time it has been mixed with the urine, and the changes which it may have suffered. If in large amount and recently shed, the urine is manifestly bloody, bright red in color, and with a sediment of corpuscles and clots. With a smaller amount of blood the color ranges from red through brownish or reddish amber to the normal color of the urine. If the blood has remained a long time in the urine, the hæmoglobin may undergo transformation

to derivative substances that give the urine more of a smoky, or brown, or dark color.

In cases of hæmaturia it is important to determine if possible the seat of the hemorrhage.

Hæmaturia originating from the *kidney* occurs in acute nephritis, congestion, chronic nephritis, especially with sclerosis and degeneration of the renal vessels, traumatisms, malignant disease, tuberculosis, conditions like purpura, scurvy, and hæmophilia, malaria, and from the action of cantharides and other poisons. The urine in cases of renal hæmaturia has the blood intimately mixed with it, and is of a darker and more smoky color than in vesical hæmaturia. Renal tube-casts may be present and the occurrence of blood casts is quite pathognomonic. Blood clots are absent or small, or may be cylindrical casts of the ureter. The red corpuscles are apt to be considerably altered, decolorized, or even disintegrated altogether.

Hæmaturia originating from the *ureter or renal pelvis* is caused by the presence or passage of calculi and other causes. From the ureter the hemorrhage is usually slight; cylindrical blood clots moulded in the ureter may be passed.

Hæmaturia originating from the *bladder* results from ulcers, cystitis, vesical calculi, malignant disease, tuberculosis, vascular tumors, rupture of varicose vesical veins, etc. In vesical hæmaturia the blood is usually less intimately mixed with the urine than in the renal forms, the color is brighter red, clots may be more abundant, larger, and irregular. Features pointing to the bladder as the seat of trouble may be present.

Hemorrhage from the *urethra* is caused by traumatisms, urethritis, neoplasms. The blood may be passed independently of the urine, or in the first portion of the urine voided. During menstruation blood is apt to find its way into the urine of women from the vagina.

Hæmaturia from various parts of the urinary tract may also originate from parasitic conditions, as from filaria, schistosoma hæmatobium, or protozoa.

Red blood corpuscles in the urine may exhibit their ordinary form, or they may be more or less altered by the action of the urine. They may be crenated, irregularly contracted or deformed, or they may become decolorized ("shadow corpuscles"); or in alkaline urine they may be destroyed and disappear.

Hæmoglobin may occur in the urine in two conditions, either alone and unaccompanied by other ingredients of the blood (hæmoglobinuria), or along with other blood elements in hæmaturia. Hæmaturia is common, hæmoglobinuria uncommon. The distinction between the two rests definitely on the presence of red corpuscles; if these are present the condition is hæmaturia, if hæmoglobin is present but the corpuscles are absent it is hæmoglobinuria; and before settling on the latter diagnosis it must be considered that red corpuscles if in small number may become disintegrated and escape detection.

Hæmoglobinuria is a result of hæmoglobinæmia, the excess of hæmoglobin in the blood plasma, derived from excessive disintegration of red blood corpuscles, being excreted by the kidneys. It is thus indicative of excessive erythrocytolysis (also perhaps of inability of the liver to transform hæmoglobin into bilirubin), and as such is rather a serious symptom. Hæmoglobinuria occurs at times after the ingestion of various poisons, transfusion of animal blood, burns, in paroxysmal hæmoglobinuria, acute fevers, etc.

In hæmaturia, hæmoglobin occurs not only in the red corpuscles, but passes into solution in the urine. Hæmoglobin in the urine imparts its red color to the fluid, and also, being a proteid, gives the reactions of albumin. Instead of hæmoglobin, derivatives from it may appear in the urine, as methæmoglobin, hæmatin, hæmatoidin, hæmatoporphyrin, and perhaps others.

Methæmoglobin is often present in the urine in the same conditions (hæmaturias and hæmoglobinurias) in which hæmoglobin appears. It may be excreted from the blood as methæmoglobin, or it may be formed from hæmoglobin in the urine. It is closely related to oxyhæmoglobin, but is of a brown color, and gives the dark color to urine containing altered blood.

Hæmatin is rarely present in the urine, arising from the transformation or decomposition of hæmoglobin (which consists of a combination of a globulin with hæmatin).

Hæmatoidin is rarely seen in urine, in connection with conditions similar to those causing hæmaturia and hæmoglobinuria. It is a derivative of hæmoglobin, is ordinarily regarded as identical with bilirubin, and occurs in undissolved crystalline or amorphous form.

Hæmatoporphyrin is formed by the action of acids on blood

pigment. It may be demonstrated in many normal urines in minute amount. Rarely it appears in large quantity (hæmatoporphyrinuria) giving the urine a brownish-red color (sherry or port-wine color); and such urines may darken on standing. Hæmatoporphyrinuria occurs especially after excessive use of sulfonal and similar drugs, in some cases of gastric and intestinal hemorrhages, at times in rheumatism and other conditions, and even after the use of food containing much hæmoglobin.

Lymph would appear in the urine (lymphuria, chyluria) from rupture of lymph vessels into the urinary tract, as in filariasis. Lymphuria would be manifested chiefly by the presence of fibrin and albumin; chyluria by the presence, also, of fat (page 153).

Pus in the urine (pyuria) enters in connection with catarrhal or suppurative processes or abscesses along or communicating with the urinary tract. It is manifested by the presence of large numbers of leucocytes, albumin, and nucleo-albumin. The pus may be intimately mixed with the urine, especially if the latter is acid, causing a general turbidity or subsiding as a white granular sediment. If the urine is ammoniacal the leucocytes disintegrate and a mucoid, viscid, tenacious, opaque white sediment is formed. It is important to determine the source of the pus.

Pus derived from the kidney or renal pelvis may be either scanty or abundant; unless the affection is bilateral the urine is usually acid and may have a characteristic odor. The presence of casts indicates renal involvement, and pus casts are quite pathognomonic of renal suppuration. The pyuria may be intermittent owing to temporary ureteral obstruction.

Urine containing pus of vesical origin is usually ammoniacal and foul.

Pus is passed with the urine in urethritis, especially in the portion of the urine first voided, the latter portion of the urine being clear or at least clearer than the first. In this condition microscopical mucous filaments studded with leucocytes ("gonorrhoeal threads") appear and are very characteristic. In women pus from leucorrhoeal discharges may become mingled with the urine.

Bile, or its characteristic constituents, occurs in the urine (choloria) in icteric conditions. The constituents of bile that appear in the urine are bilirubin (and its derivatives), bile salts,

cholesterin, and nucleo-albumin. Urine containing bile elements is very characteristic in its appearance and properties; it is of a clear, deep amber or brown color, very frothy upon being shaken, the foam also being golden or brown in color, and such urine leaves a yellow stain on paper or cloth.

Bilirubin, the primary bile pigment, is diagnostically the most important and conspicuous element of the bile in urine, on which the diagnosis of choluria is made. It imparts to such urine a characteristic deep amber or brown color. Under the action of oxidizing agents, as nitrous acid, hydrogen peroxide, and others, bilirubin is converted successively into a series of pigments exhibiting a marked contrast of colors. The rich amber bilirubin is first transformed into biliverdin, a bright-green pigment; this in turn changes into a blue substance, followed by a purplish-red, and then, it is said, a yellow substance; on these reactions are based the tests for bilirubin. The pigment excreted into the urine is bilirubin; such urine on standing may turn green from the formation of biliverdin. Bilirubin very rarely appears in urine in undissolved crystalline or amorphous form.

Altered Bilirubin.—It occasionally happens that the urine from icteric patients, although presenting precisely the same color and other characteristics that are shown by urine containing normal bile pigment, does not give the reactions of bilirubin. Instead of green, a red, brownish, or even blackish color appears on treatment with nitric or other mineral acids. In these cases the bilirubin has undergone some slight alteration, of obscure nature, to a substance incapable of oxidation to biliverdin. This altered bilirubin is perhaps related to the pigment of febrile and similar urines.

Bile salts and acids appear in urine associated with bilirubin in icterus and possibly under other circumstances. Their demonstration is difficult.

Cholesterin, in the form of undissolved crystals, has been observed in the urine in a very few cases in chyluria and nephritis (not in jaundice).

Diazo Reaction.—This is a color reaction of the urine obtainable in certain conditions by means of diazo-benzene-sulphonic acid. The substance which yields the reaction is entirely unknown. The diazo reaction usually occurs in typhoid fever, measles, acute tuberculosis, severe stages and terminal stages of chronic

tuberculosis, and perhaps in smallpox; only exceptionally in other diseases, acute or chronic. It is of some diagnostic and prognostic value, and worthy of clinical consideration, especially in typhoid fever. The reaction usually appears at some time in the course of typhoid fever, and often early enough (during the first week) to be of great significance. Its absence does not, however, negative the diagnosis.

Toxins.—Toxic substances varying in amount and kind are excreted in the urine in both normal and abnormal conditions. They originate from normal or abnormal katabolism (leucomains), in the intestine, as products of bacterial growth in infectious conditions, or products of decomposition. They are mostly alkaloidal bodies of the nature of ptomains or diamines, some of which have been isolated, as putrescin, cadaverin, etc. A constitutional tendency to the formation and excretion of certain diamines sometimes appears to be associated with cystinuria.

Toxicity of the Urine.—The poisonous ingredients of the urine impart to it various toxic properties, as myosis, dyspnoea, narcosis, diuresis, salivation, hypopyrexia, convulsions, etc. The toxic powers of the urine vary in kind and degree in different conditions, and are determined by intravenous injection of the urine into rabbits or other animals; the amount required to produce special symptoms or to cause death furnishes a quantitative index of the toxicity of the urine. Animals are killed by normal urine injected intravenously in the average proportion of 45 cubic centimetres of urine to 1 kilogram of body weight. The toxicity of the urine is diminished (larger amounts being required to kill) when the excretory power of the kidney is impaired (as in uræmia), the toxins not being excreted. Its toxicity is increased or new toxic symptoms are produced, in most acute infectious diseases, and leukæmia, unless the functional power of the kidneys is impaired. The urine in such cases (as in tetanus and cholera) may reproduce the prominent phenomena of the disease itself.

As the urine in typhoid fever may yield the Widal reaction, it is evident that agglutinins as well as other bacterial products are excreted by the urine.

Adventitious Ingesta.—Many food and drug substances introduced into the system by the alimentary tract or otherwise are

excreted in the urine, either in their original or a modified form. Some of these affect the appearance of the urine, some become evident during the application of the usual urinary tests, others may be demonstrated by special methods. At times diagnostic information of value is afforded as to poisons ingested.

The ingestion of rhubarb, senna, or santonin turns the urine bright yellow if acid, or red if alkaline. Various dyes, as methylene blue, indigo, madder, etc., impart to the urine their respective colors. Salicylic acid is excreted as salicyluric acid, which if in large amount colors the urine green or smoky. Urines containing the derivatives excreted after free ingestion of phenol are green or greenish-black, and turn darker on standing. Excreted iodides form a sort of chromogen, yielding the brown color of free iodine on adding nitric acid. Most of the alkaline salts of organic acids are excreted as carbonates. After the ingestion of drugs like copaiba and turpentine the resinous substance is excreted in the urine and in the nitric-acid tests appears as a white precipitate, which, however, differs from albumin in being soluble on the addition of alcohol. A case is recorded in which coal dust temporarily appeared in the urine in the case of a cleaner of stoves.

Epithelium cells desquamated from the urinary passages are usually present in the urine, varying from very scanty numbers to a quantity sufficient to cause general cloudiness. Large numbers of cells also enter from the vagina, so that the cells are usually much more numerous in the urine of women than of men. The cells may be separate and single, or attached together in as yet unbroken aggregations.

As to the form, the epithelial cells in the urine can be classed as squamous, spherical, and elongated or irregular.

The **squamous cells** are large, expanded, flat pavement cells. The body protoplasm is more or less granular, and in the centre is a round granular nucleus; sometimes two nuclei are present. The cells are often curled or rolled up. Cells of this kind are desquamated from the most superficial layers of the renal pelvis, ureters, bladder, and vagina, and have little or no pathological significance.

The **spherical cells** frequently seen in urine, smaller than the flat cells, are rounded or spheroidal in shape, finely granular, each with a large central spherical nucleus. They differ from

leucocytes in their larger size and single round nucleus. Some of them may originate from the renal tubules, such as the cells forming epithelial casts; they are mostly, however, derived from the deeper or germinal layers of the epithelial lining of the renal sinus, ureters, and bladder. Those of recognizably renal origin in connection with other renal symptoms indicate disease of the kidney. Those derived from the urinary passages, being cast off rather prematurely in immature form, to that extent indicate slight irritation or catarrh; in small number, their significance in this respect is trivial, but in large numbers they may accompany catarrhal conditions.

The term *mucous corpuscle* is used in urinological literature in a rather vague sense; if used at all, it is best applied to designate the spherical epithelium cells cast off immaturely from the germinal layers of the urinary mucosa.

Elongated, columnar, fusiform, pyriform, or irregular cells sometimes appear in the urine; their origin and significance are simi-

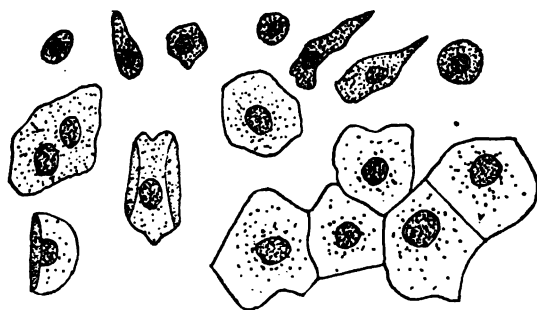


FIG. 26.—Epithelium Cells in Urine.

lar to those of the spherical cells, and they are derived from the kidney or from the deeper layers of the epithelial lining of the urinary passages.

It is not often possible to determine with certainty, from the form of the epithelial cells, the precise region from which they are derived. Precisely the same kind of epithelium lines the renal pelvis, ureters, and bladder; the squamous cells of the vagina cannot practically be distinguished from those of the urinary passages; and round or elongated cells from the kidney, when not in casts and after being altered by the urine, are practically identical in appearance with those from the urinary passages.

Fragments of tissue, as neoplasms, are rarely passed with the urine and may afford valuable diagnostic information.

Leucocytes are frequently present in small numbers in normal urine, with no clinical significance, being wandering cells that have worked their way through the tissues. They are present in increased number in local inflammations, hæmaturia, lymphuria, and in very large number in the severe inflammatory, catarrhal, and suppurative conditions of the urinary organs attended with pyuria. They may also be derived from vaginal discharges. They are mostly of the polynuclear variety; sometimes exhibit amœboid movements; may become altered or disintegrated by the action of the urine, especially if ammoniacal; and by strong alkalies are converted into a viscid mucoid mass.

Spermatozoa appear in the urine after coitus, masturbation, or emission from other causes, in spermatorrhœa, and sometimes after epileptic convulsions or in other local or general affections. They may appear in the urine of women after coitus.

Casts.—Tube casts or cylinders are moulds or casts formed in the renal uriniferous tubules, and are of great diagnostic significance, being almost always distinctly pathological and always at least suspicious. They are long, cylindrical bodies, 20 to 50 micromillimetres in diameter, unbranched, usually straight, but sometimes irregularly curved as if following the course of a convoluted tubule. They are composed of various materials, according to which they are classified as follows: hyaline casts, epithelial casts, granular casts, fatty casts, waxy casts, leucocyte casts, blood casts, bacterial casts, urate casts. Cyliindroids are objects somewhat similar to the casts.

Hyaline casts are the forms most frequently seen. They are pale, colorless, clear, hyaline, and faintly outlined in the microscopic fields. Sometimes they contain scattered granules or a few epithelium cells, or a portion of the casts may be granular or epithelial, indicating a transition to granular and epithelial casts. Granules, crystals, or cells may also become attached to their surface subsequent to their discharge from the kidney. They seem to be formed by proteid material from the blood becoming coagulated in the tubules and thus forming a mould of them. They are abundant in acute and chronic diseases of the kidney, and are strongly indicative of renal congestion, acute or chronic nephritis, or degeneration, especially when, as is usually

the case, they are accompanied by albuminuria and other renal symptoms. At times, however, they appear in the urine in very small numbers without concomitant albuminuria and in individuals without demonstrable renal or other lesions.

Epithelial casts are moulds of the tubules formed of the cells of the tubes cemented together by hyaline material. The cells may exhibit their original form, little changed, with nuclei distinct, or they may be degenerated, becoming granular or fatty. They indicate renal disease of the same nature as do hyaline casts.

Granular casts are composed of granules, coarse or fine, dark or light colored, cemented together in the form of tubules. The

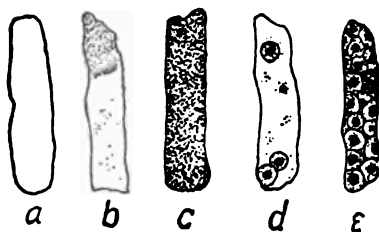


FIG. 27.—Casts from Urine. *a*, Hyaline cast; *b*, hyalo-granular cast; *c*, granular cast; *d*, hyalo-epithelial cast; *e*, epithelial cast.

granular material is derived from the débris of broken-down epithelium, blood, or pus cells. Their significance is the same as that of hyaline and epithelial casts.

Fatty casts originate from epithelium cells that have undergone extensive fatty degeneration, and contain abundant fatty globules. Their

surface may exhibit fatty-acid crystals. They indicate fatty degeneration of the kidney.

Waxy casts resemble the hyaline casts, but are larger, rather yellowish, more refractile, more conspicuous, and more substantial in appearance than the hyaline casts. Sometimes they yield the amyloid reaction with iodine, sometimes not. They are uncommon. Their mode of origin and significance are not definitely settled. They have no exclusive association with amyloid disease of the kidney; that is, they occur in other conditions as well as amyloid kidney, and do not occur in all cases of amyloid kidney.

Leucocyte casts, or pus casts, are composed of leucocytes cemented together in the form of casts. They are rare, occurring in suppurative conditions of the renal parenchyma.

Blood casts consist of, or contain, red blood corpuscles, and are indicative of hemorrhagic conditions of the renal substance. They are rare. Casts composed of granules of hæmatoidin or hæmoglobin have been observed in cases of hæmoglobinuria.

Bacterial casts, consisting of an aggregation of bacteria, may occur in infectious, septic, or embolic conditions of the kidney, and are of serious import.

Urate casts, composed of amorphous urates cemented together, may appear in the urine of new-born infants, and in cases of gouty kidney.

Cylindroids are long, pale, hyaline, faint band-like formations somewhat similar to, and indeed at times difficult to distinguish from, hyaline casts. They are of greater length than the casts; at one end they taper to a point or filament, and at the place where they taper usually exhibit a characteristic indented or scalloped outline. They are generally regarded as being composed

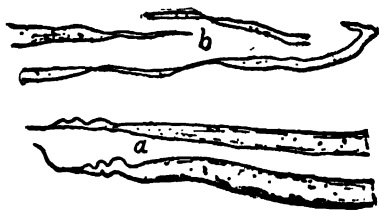


FIG. 28.—Cylindroids (a) and Mucous Threads (b).

of mucoid material, and as being formed within the uriniferous tubules. Granules and cells may be adherent to them. They are frequently associated with mucous threads, and at times with hyaline casts. They are frequently observed and are not of much pathological significance, only a slight degree of renal irritation being necessary to produce them.

Mucous threads are fine, colorless filaments, long and slender, often matted and tangled together; they consist of the trace of nucleo-albumin or mucin in the urine precipitated in threads by the action of the acid principles of the urine, and appear most markedly in highly acid and concentrated urines.

Spirals analogous to Curschmann's spirals of the sputum have been observed in the urine in one or two instances.

Granular and amorphous debris may be present in the urinary sediment, derived from broken-down cellular material or insoluble chemical constituents.

Calculi.—The commonest calculi derived from the urinary organs, kidney or bladder, are composed of earthy phosphates, calcium oxalate, uric acid, or urates. Very rarely urinary calculi are composed of xanthin, indigo, cystin, calcium carbonate, fat or soap (urostealith), cholesterin. Prostatic and urethral calculi are sometimes encountered, as well as renal and vesical concretions.

Parasites.—The organisms appearing in the urine are both animal and vegetable.

Animal Parasites.—Three or four species of protozoa, trichomonas, amœbæ, and others, have in a few instances been observed in the urine. Trichomonas is probably the commonest, usually wandering into the bladder or urethra from the vagina. Some of these parasites are harmless, others more or less pathogenic, being especially associated with hæmaturia.

Vermes: *Schistosoma hæmatobium* is common in Egypt and certain other localities; the parasite inhabits the blood-vessels in the vicinity of the urinary organs, and the ova and sometimes the embryos appear in the urine, along with blood. Larval *filariæ* may appear in the urine, with chyluria and hæmaturia, in filariasis. In hydatids of the urinary organs, the cysts, hooklets, or portions of the larval *echinococcus* may be voided with the urine. *Diectophyme renale*, a large nematode, 30 to 100 centimetres in length, usually occupying the pelvis of the kidney, has occurred in man in a very few cases, it or its ova appearing in the urine. Intestinal parasites, as ascaris, very rarely find their way into the bladder through intestinal fistulæ. The larval arachnoid pentastoma denticulatum has been observed in the urine.

Animal parasites are exceedingly rare in the urine in this country.

Vegetable parasites of many species and in large numbers are frequently present in urine, but in the great majority of cases they are introduced and develop rapidly after the urine is voided. Certain species are capable of causing marked changes in urine, as those producing ammoniacal transformation of urea, alcoholic or acid fermentation of diabetic urine, pneumaturia or hydrothionuria. The vegetable micro-organisms that may be present in the urine when voided originate from the urethra, the bladder, ureter, or renal sinus, or from the renal parenchyma; and they may be either pathogenic or innocuous. Bacteria are apt to be normally present in the urethra. In the bladder they are ordinarily absent, though at times a few may be there present without causing trouble. They are present in large numbers in the bladder, ureters, or renal sinuses in infected or inflammatory conditions of these passages or in vesical atony and stagnation; in such cases they usually cause decomposition of the

urine while in the bladder. From the kidney substance bacteria may be derived from infected areas of the organ or by a process of excretion in systemic infections.

The vegetable parasites of the urine belong to the fungi and bacteria.

The higher fungi found in the urine are mostly contaminations subsequent to its being voided. Rarely pathogenic fungi, as actinomyces, aspergilli, etc., are derived from the urinary organs themselves. The fungi appear on microscopical examination in the form of mycelia or stellate forms. Sacccharomycetes or yeast fungi do not often appear in the urine; they are most apt to be present in glycosuria.

Bacteria are the most abundant and most important organisms in the urine. Many species, some pathogenic, some causing fermentation, some harmless, are frequently present, though not normally to any material degree in freshly passed urine. The pathogenic bacteria are chiefly derived from local infections of the genito-urinary organs, such as the gonococcus, tubercle bacillus, the ordinary pyogenic bacteria (staphylococci, streptococci, colon bacilli), and these or other micro-organisms concerned in the causation of cystitis, pyelitis, etc. In some general infectious diseases the causative bacteria may be excreted into the urine through the kidneys, without there being any local infection of the urinary organs by the germs; thus in typhoid fever, croupous pneumonia, erysipelas, etc, the typhoid bacilli, pneumococci, streptococci, etc., may appear in the urine without there being any nephritis, pyelitis, or cystitis present. Rarely bacteria are voided in the urine in large numbers without any local or general infection of consequence being demonstrable ("idiopathic bacteriuria").

The determination of pathogenic bacteria in the urine may afford information of diagnostic value.

Foreign Bodies.—Extraneous objects, as fibres, particles of various kinds, etc., are apt to appear in the urinary sediment, varying in amount according to the cleanliness of the parts or the receptacles. Starch granules from toilet powders used on the genitals (female) are sometimes abundant. Such extraneous bodies should not be allowed to cause error.

In cases of vesico-intestinal fistula faecal matter may be voided with the urine; such an occurrence is diagnostic.

B. PHYSICAL CHARACTERS OF URINE.

The physical characters of the urine depend on its chemical composition, and a consideration of these characters yields important information.

The quantity of urine passed daily is normally about 1,000 to 1,500 cubic centimetres, but fluctuates greatly according to the amount of water excreted. It is increased (polyuria) after free ingestion of liquids, when perspiration is not active, under the use of diuretics, in diabetes mellitus and insipidus, chronic interstitial nephritis, certain nervous and other conditions.

It is decreased (oliguria) with lessened drinking of fluid, with increased perspiration, in hepatic diseases (cirrhosis, acute yellow atrophy, yellow fever), in most renal affections, in acute fevers. The daily amount of urine is an important item for clinical consideration, since without it no adequate idea can be formed as to the amount of excreted products. Complete suppression (anuria) may occur.

Specific Gravity.—This depends upon the relative proportion of dissolved solids, and for the most part varies inversely to the fluctuations of the water. It is normally from about 1.013 to 1.025 (pure water being 1.000). It is lowered in most conditions of polyuria and hydruria, ranging down to 1.000; it is increased in concentrated urines, as in fever, and in diabetes mellitus. The greatest increase of specific gravity occurs in diabetes mellitus, not infrequently exceeding 1.040, though exceptionally glucose is present in urines of specific gravity as low as 1.012. Concentrated urines other than diabetic do not often exceed a specific gravity of 1.035.

Total Solids.—The total solids excreted daily in the urine amount normally to about 60 or 70 grams, of which the most abundant ingredients are the following:

Urea.....	about 30 grams.
Other nitrogenous bodies.....	8 "
Pigments.....	6 "
Chlorides.....	15 "
Phosphates.....	5 "
Sulphates.....	3 "

The amount fluctuates according to the ingesta, metabolic activity, renal sufficiency, and the presence of abnormal substances (glucose, albumin).

The **undissolved solids** of the urine merit special consideration since as a distinct group they affect the appearance of the urine (its transparency) and require special methods of examination (microscopical). When present to the extent of about .2 per cent by bulk, or more, the undissolved particulate solids in the urine are sufficient to cause general cloudiness. In fresh normal acid urine the undissolved solid bodies are extremely scant, consisting of little more than a few scattered epithelium cells. After standing a light flocculent cloud or *nubecula* may appear in acid urine, and gradually settle to the bottom; this is formed by delicate mucous threads precipitated by the acid principles, with which may be entangled cells, granules, or crystals. Acid urines in the cold (as in winter or in an ice-box) deposit an abundant white or pink granular sediment of urates, or if strongly acid a more scanty sediment of uric-acid crystals appearing like red-pepper grains. Alkaline urines usually exhibit a turbidity or sediment of pale, granular earthy or triple phosphates. The development of large numbers of bacteria causes a diffuse cloudiness; the bacteria do not settle to the bottom, even with the centrifuge, and cannot be removed with filter-paper.

The occurrence of various abnormal substances, as pus, leucocytes, blood corpuscles, clots, an excess of epithelium, spermatozoa, or calcium oxalate, and others less common, may produce cloudiness or sediments. In lipuria, the oil present rises to the top.

Transparency.—Fresh normal acid urine is perfectly clear and transparent. The urine becomes cloudy when undissolved solids are diffused through it, the turbidity varying from slight cloudiness to dense opacity. Turbidity of alkaline or weakly acid urine, increasing with heat, and becoming clear on the addition of a drop or two of acid, is due to earthy phosphates. Turbidity of acid urine, clearing with heat or addition of alkali, is due to urates. Other causes of turbidity will be revealed by microscopical examination. Most turbid urines become perfectly clear on filtration, and clear or nearly so after settling; the cloudiness caused by bacteria is practically undiminished by ordinary filtration, and does not clear by sedimentation.

The **sediment** in urine also depends entirely on undissolved solids, and is associated with cloudiness of the urine, the sub-

stances causing turbidity usually settling to the bottom as a sediment.

The reaction of the urine may be acid or alkaline, in varying degree, neutral, or amphoteric, according as acid or alkaline principles preponderate or balance each other. Not only the kind of reaction, but also in many cases the degree of acidity or alkalinity is clinically important, as whether the urine is strongly, moderately, or slightly acid; by titration the degree of acidity can be determined with quantitative precision.

The chief acid principles of the urine are the acid phosphates of sodium and potassium and carbon dioxide; rarely free fatty acids also. The principal substances giving urine an alkaline reaction are the carbonates and neutral phosphates of sodium, potassium, and ammonium. Of these, carbon dioxide and ammonia compounds are volatile; they are dissipated by heating, correspondingly affecting the quantitative reaction of the urine; and litmus paper changed by them resumes its original color again on drying. The sodium and potassium compounds are "fixed" and non-volatile acid and alkaline principles.

The mixed twenty-four-hour urine is normally moderately acid; individual passages may be neutral or alkaline (from fixed alkali) without being materially abnormal. The acidity is increased after the ingestion of acids, meat diet, and muscular exercise, in concentrated urines, gouty and other conditions, certain neurotic cases, and after acid fermentations. Its acidity is diminished or it may become alkaline from the ingestion of vegetable foods and certain drugs, as a result of mental work or nervous influences, or ammoniacal fermentation. The degree of acidity fluctuates at different times of the day, diminishing after meals. The quantitative acidity of the normal twenty-four-hour urine ranges from about 10 to 40, averaging about 25.

The color of the urine normally is yellow, ranging from light yellow to bright amber. In concentrated and febrile urines the color is a deep amber to reddish. In dilute urines of hydruria and polyuria the color is paler, ranging down to nearly colorless. In anæmic conditions the color is paler, owing to the deficiency of hæmoglobin from which the urinary pigment is ultimately derived.

Various alterations of color are produced by abnormal ingredients. Bile pigments produce a rich deep or brownish amber; on standing such urines occasionally turn green. Hæmoglobin

and its derivatives impart a red, brown, or dark color. Hæmatoporphyrin produces a brownish-red color, darkening on standing. Melanin imparts a dark color, or darkens on standing. Indican is ordinarily colorless, but on decomposition rarely turns urine red or blue. Urines containing alkapton or derivatives of phenol may darken on standing. Various drugs and food-stuffs impart their color to the urine. In turbid urines the color is modified by the undissolved solids present; thus, urates cause a dirty yellow color, which on warming or filtering becomes a normal amber; chylous urine is white and milky.

Urine, especially ammoniacal, is fluorescent.

Consistency.—Normal urine is thin and fluid like water. A large proportion of solids, especially albumin or glucose, makes it slightly less fluid. An abundance of mucin, pus, or fibrin makes urine much thicker, sometimes fairly gelatinous. Chylous urine is also thick.

Frothiness.—After agitating normal urine a certain amount of froth appears on the surface which subsides more or less slowly. The frothiness is greater in concentrated urines, less in dilute urines. It is much increased in urine containing albumin, mucin, or bile. Its causes and variations are the same as those of the emulsifying action of urine.

Emulsifying Properties.—On agitating urine with a fluid not miscible with pure water, as chloroform, ether, or oil, it will be found after the liquids separate that the oleaginous fluid remains coarsely emulsified to a variable extent, being either broken up into globules through its entire mass or with an emulsified layer at the contact zone. Normal urine possesses a distinct and sometimes marked emulsifying power over such liquids; this action is increased in concentrated urines, diminished or abolished in dilute urines, and disappears after the urine is treated with lead acetate or decolorized with *carbo animalis*. Albuminous urine possesses strong emulsifying power.

The emulsifying action of normal urine has been by some observers regarded, erroneously, as an evidence of the presence of minute amounts of albumin in normal urine. Albumin in such dilution as to give no response to the ordinary tests possesses no material emulsifying power; this power in normal urine is therefore not due to, or a proof of, the presence of albumin, but is caused by other substances, perhaps urochrome.

The odor of normal urine is characteristic; it increases or diminishes according to the density of the urine. Ammoniacal and decomposing urine has a foul odor, acetone imparts a fruity odor, hydrogen sulphide a disagreeable odor. In pyelitis the urine may have a peculiar offensive odor. Some foods and drugs impart special odor to the urine, as asafoetida, turpentine, copaiba, asparagus, garlic.

The freezing point of urine varies according to the presence of certain organic material, so that determination of the freezing point (cryoscopy) has recently been utilized for the purpose of obtaining information as to these substances.

Variation of Urine.—The composition of the urine varies from time to time during the twenty-four hours, according to body activity and ingestion of food. No one passage is exactly representative of the entire day's urine. Albumin, sugar, urea, etc., are increased after exercise or meals, while they are often diminished or even absent in the morning urine. After meals the acidity of the urine is decreased. During some conditions, as the passage of renal calculi, or at the crisis of acute diseases, the urine may change markedly in the course of a few hours.

Permeability of the Kidneys.—By this is meant the time elapsing after the ingestion of various substances until they appear in the urine, and again until they are entirely excreted and disappear from the urine. Methylene blue, potassium iodide, phloridzin, and similar easily recognizable substances are employed for test purposes. The time to the beginning and end of excretion represents the functional activity and quickness of the kidney. Normally, methylene blue appears in the urine within one-half to two hours, and is entirely eliminated by the third day. In interstitial nephritis the methylene blue often does not appear for twenty-four hours, and is not entirely eliminated until about the fifth day.

Changes on Standing.—With the lapse of time urine is prone to undergo a variety of changes. Such changes may occur while the urine is still in the bladder, in infected, paralytic, atonic, or obstructive conditions causing stagnation of the urine in this viscus; or they may develop after the urine is voided. It is therefore necessary to examine urine as fresh as possible, both to determine what changes it may have undergone in the bladder

and to avoid being misled by subsequent alterations, which are often needlessly alarming to patients.

Nubecula-formation.—The appearance of a light cloud of tangled mucoid threads shortly after passage of urine, especially if strongly acid, is a normal occurrence.

Bacterial Growth.—Bacteria are very prone to develop in urine. This may occur in the bladder in infected conditions of the organ; or urine passed clear may very quickly become contaminated and clouded by enormous bacterial growth, especially in warm weather. Certain bacteria further cause marked transformation, fermentation, or decomposition of the urinary constituents.

Ammoniacal fermentation is one of the commonest changes to which urine is subject under the action of contaminating bacteria. It very frequently occurs in the bladder, and is one of the chief features of cystitis or urinary stagnation in this organ; it may also develop in urine subsequent to its passage. It is caused by certain bacteria which have the power of changing urea to ammonium carbonate, thus, $\text{CON}_2\text{H}_4 + (\text{H}_2\text{O})_2 = (\text{NH}_4)_2\text{CO}_3$. Urine undergoing ammoniacal fermentation is very distinctive in its characteristics. It has a foul ammoniacal odor; it is strongly alkaline, turbid from the precipitation of earthy phosphates, effervesces with acid from the presence of carbonates, and under the microscope shows large amounts of granular amorphous phosphates, numerous ammonio-magnesian phosphate crystals, many bacteria, and sometimes calcium carbonate and spherules of ammonium urate.

Carbohydrate Fermentation.—Exceptionally urine containing glucose or other carbohydrates may undergo fermentation, of either the alcoholic or lactic-acid form, under the influence of micro-organisms of the yeast or lactic-acid bacillus type respectively. This may occur in the bladder or subsequent to micturition.

Effect of Cold.—After being voided, urine exposed to cold (as in winter) deposits its urates as an abundant sediment.

Some urines after standing for a sufficiently long time deposit crystals of uric acid. The nature and cause of this deposition, and the associated changes in the other urinary constituents, are not well understood.

Urines containing hæmatoporphyrin, alkapton, or derivatives of phenol darken on standing. Urine containing bilirubin some-

times turns green, while decomposition of indican may cause urine rarely to turn blue or red. Decomposition of cystin generates hydrogen sulphide.

Action on Polarized Light.—Normal urine rotates polarized light slightly to the left (10°). Polarized light is rotated toward the left by indican if in sufficient amount, serum albumin (56°), other proteids, levulose (106°), glycuronates, and β -oxybutyric acid. It is rotated toward the right by glucose (56°), lactose (59°), sucrose (74°), dextrin (138°), maltose (150°), glycogen (211°), free glycuronic acid, and some pentoses. Certain drugs or their derivatives excreted into the urine also affect polarized light.

C. EXAMINATION OF URINE.

The examination of the urine comprises a variety of procedures—simple inspection, physical, chemical, microscopical, etc. The large number of ingredients of the urine requires a correspondingly large number of processes for the detection and estimation of them all; only a few of these substances are, however, considered in ordinary clinical work, and the limits of this work permit only the most important and most frequently used procedures of urine analysis to be detailed.

Some such blank as that on the following page will be found convenient for recording and reporting the results of urine examination.

Collection of Specimens of Urine.—In examining urine it is usually by far the most satisfactory to use a sample from the entire passage of the twenty-four hours mixed together. The urine may vary considerably during the day, and no one passage can be taken as representative of the entire twenty-four-hour urine. That passed after meals or exercise contains an amount of katabolic and ingested products above the average, while that of the morning is below the average. Of all the passages of urine during the day, that of the morning on arising is least representative and satisfactory; for instance, in slight albuminuria or glycosuria, the repose of the night may cause a disappearance of albumin or sugar from the urine, when it would appear after exercise. The collection of the urine for the entire day also affords a means of measuring the amount for the twenty-four

Date, ..., 190 .

EXAMINATION OF URINE.

Name of patient.....	
Amount in 24 hours.....	c. c.
Color.....	Transparency
Reaction.....	Specific gravity (at 15° C.)
Albumin.....	
Sugar.....	per cent; grams in 24 hours.
Urea.....	per cent; grams in 24 hours,
Uric acid.....	per cent; grams in 24 hours.
Hæmoglobin.....	Indican.....
Bile pigment.....	Diazo reaction.....
.....	
.....	
.....	
.....	
.....	
Undissolved solids.....	

Microscopical examination:

Casts
.....
Cylindroids Mucous threads
Epithelium.....
.....
Leucocytes..... Red blood corpuscles
Bacteria Spermatozoa... ..
Uric acid.....
Urates.....
Phosphates.....
Calcium oxalate.....
.....
.....
.....
.....
.....
.....

(Signature)

hours, which in itself is a valuable clinical datum, and enables the daily amount of the individual excreta to be determined.

To collect the twenty-four-hour urine, the bladder should be emptied at a given hour, say 7 A.M., and the urine thus passed *thrown away*. All the urine voided in the ensuing twenty-four hours, including that passed at stool, should then be scrupulously saved in a clean, large, stoppered bottle or jar. At the stated hour on the following day the bladder should be again emptied, and this portion of the urine *saved* and added to the rest. By this means the renal secretion for exactly twenty-four hours is collected. By the addition of phenol or other antiseptic, bacterial changes will be prevented.

In some cases in which rapid changes are in progress, as during the passage of a renal calculus or other obstruction of the ureter, or at the crisis of acute disease, it may sometimes be useful to examine the urine at short intervals (every few hours) in order to follow the changes going on.

In order to distinguish between vesical and urethral affections, the first part of the urine voided at a micturition may be passed in one receptacle, and the last part in another. If the first portion contains pus or other abnormal elements while the second part contains less or none, disease of the urethra is indicated. If the second portion is more abnormal than the first, disease of the bladder is indicated.

It is sometimes useful to obtain urine secreted by each kidney separately, in order to ascertain in case of unilateral disease which kidney is affected, or whether either kidney is absent or its outlet obstructed, or whether the kidneys are affected at all. In the case of women an expert gynaecologist can catheterize the ureters and thus obtain a sufficient amount of the secretion of each kidney separately for test purposes. With men catheterization of the ureters is scarcely practicable. Instruments have, however, been devised for introduction into the bladder to collect separately the secretion from the two sides.

Preservation of Urine.—When urine cannot be examined fresh, or in collecting the twenty-four-hour urine in warm weather, bacterial growth and chemical changes can be prevented by the addition of preservatives or antiseptics, such as phenol, of which three to five drops may be used for every hundred cubic centimetres of urine. Some of these to a slight degree reduce copper

salts, but do not afford other glucose reactions. The urine may also be kept in the cold; this precipitates urates, but these may be redissolved by warming at the time of examination. Antiseptics should not be added to urine that is to be subjected to the fermentation test for sugar.

Clarification of Urine.—For many purposes in urine analysis it is not necessary that turbid urine be clarified; but in some instances, as in testing for albumin, it is important that the urine be clear. Turbid urine can usually be clarified by filtering; but sometimes, especially if the cloudiness is due to bacteria, ordinary filter-paper will not clear the urine. In such cases, filtration after adding insoluble substances like talc, chalk, magnesia, etc., or after adding alkali, lime water, etc., to precipitate phosphates, may clarify the fluid; this procedure, however, also removes a portion of any albumin that may be present, or if in small amount may remove it all. Urine can be completely clarified by the use of Chamberland's filter.

Urine may sometimes be clarified by allowing the sediment to settle or by throwing it down with the centrifuge. Phosphatic turbidity is cleared by adding a little acid; turbidity due to urates may be cleared by heating.

At times the color of the urine is so dark as to interfere with some of the color tests. This may be obviated by diluting it, or by decolorizing it with lead acetate or charcoal. A strong solution of neutral lead acetate added freely to urine precipitates the chlorides, phosphates, sulphates, carbonates, the normal urinary pigments (not indican), bilirubin, and all or a part of albumin present, leaving the fluid colorless. Urine shaken with a large proportion of powdered animal charcoal, allowed to stand a few minutes, and then filtered, is decolorized, the pigments and a part or all of the albumin present being removed.

Quantity.—This is determined by measurement. The method of obtaining the exact twenty-four-hour product of the kidneys has been given above.

Color.—This is noted by simple inspection, no exact colorimetric methods or uniform scale of color notation having yet come into general use. In practice, each observer adopts a scheme of color notation of his own, and different observers frequently do not employ the same terms to denote the same shades of color, and *vice versa*; so that the terms of different observers (as straw-

color, amber, etc.) do not always mean precisely the same shade. The intensity of the color varies according to the depth of the urine looked through, and here again each observer adopts his own standard.

If the urine is turbid, the color of both the unfiltered fluid and the clear filtrate may be noted.

The **transparency** of the urine,—whether it is clear, or if cloudy the degree and cause of turbidity,—should be noted.

Undissolved Solids.—These, if present, may be noted both with regard to the character and degree of the *turbidity* which they produce, and with regard to the appearance, nature, and amount of the *sediment* which collects after settling or the use of the centrifuge. The nature of the undissolved solids is determined chiefly by microscopical methods. If in the routine centrifugation a graduated tube be used the proportionate bulk of the sediment thrown down (as $\frac{1}{100}$, $\frac{1}{50}$) may be noted; this affords a measure of the undissolved solids, useful for purposes of record or comparison.

Centrifugation.—The centrifugal machine has been alluded to in a general way (page 9). In urine analysis two (or four) glass tubes of 10 or 15 cubic centimetres capacity, with conical bottoms, and (for some purposes) graduated to tenths of cubic centimetres, are employed as the urine containers (Fig. 29). The tubes should be filled alike, to preserve an even balance. Undissolved solids or various precipitates can be thrown down as a compact sediment, and their relative bulk can be determined from the graduations.



FIG. 29.—
Urine
Centrifugal
Tube.
(Bausch
& Lomb.)

The **reaction** of urine is determined by means of blue or red litmus paper. According to the intensity of the change of color of the test paper, the degree of acidity or alkalinity can be roughly judged and noted, as weakly, moderately, or strongly acid or alkaline.

Sometimes it is useful to determine the acidity of urine quantitatively. This can be done most easily by titrating a measured amount of urine with decinormal sodium-hydrate solution, using phenolphthalein as an indicator, in the same way as in determining the acidity of gastric fluid (page 89). The color of urine interferes somewhat with the delicacy of the end reaction with phenol-

phthalein, but ordinarily the results are sufficiently reliable for practical purposes.

The **specific gravity** of urine is ordinarily taken by means of the so-called "urinometer" (Fig. 30). This floats at different depths, varying according to the specific gravity of the fluid, which is shown by means of a scale graduated from 1.000 to 1.040 or more (1.000 being the specific gravity of pure water). The specific gravity of the urine is indicated by that marking of the scale which is even with the upper level of the urine. The reading should be made from just above the surface of the urine, and not from below, froth being removed. The portion of the instrument that is immersed in the urine should have no air bubbles adherent to it, and the stem above the surface of the fluid should be dry, in order not to falsify the reading. The urinometer jar, or cylindrical container for the urine, should be large enough to permit the urinometer free play up and down. The urinometer should be one carefully graduated and standardized, otherwise the results will be unreliable.



FIG. 30.—Urinometer and Jar. (Bausch & Lomb.)

If the quantity of urine available is too small to float the urinometer, it should be diluted to a sufficient and known degree, and the specific gravity of the diluted fluid multiplied by the degree of dilution to give the density of the original urine; this procedure should be employed only when absolutely necessary, as the error of reading is multiplied.

The specific gravity varies materially with the temperature of the fluid, so that urine cooled in an ice-box will give a specific gravity reading about six points higher than the same urine at body temperature. For accurate work the specific gravity observed at any temperature should be corrected or reduced to some standard temperature. The urinometers sold are graduated for definite temperatures, usually either 25° or 15° C.

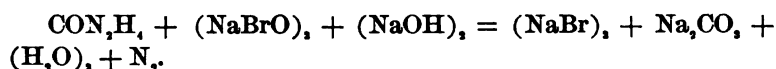
The corrections to be made vary with the temperature and also with the density of the urine, but are approximately as follows:

Temperature at which observation is made.	CORRECTION TO BE APPLIED TO OBSERVED SPECIFIC GRAVITY TO REDUCE IT TO—		Temperature at which observation is made.	CORRECTION TO BE APPLIED TO OBSERVED SPECIFIC GRAVITY TO REDUCE IT TO—	
	15° C.	25° C.		15° C.	25° C.
0° C.....	— 0.8	— 2.8	21° C.....	+ 1.0	— 1.0
10.....	— .7	— 2.7	25.....	+ 2.	0.
12.....	— .4	— 2.4	29.....	+ 3.	+ 1.
15.....	.0	— 2.0	32.....	+ 4.	+ 2.
18.....	+ .5	— 1.5	35.....	+ 5.	+ 3.

For very accurate determination of the specific gravity, the pycnometric method may be used, the weight of any accurately measured volume of urine being obtained with delicate scales. From these data the specific gravity is calculated, and reduced to standard temperature.

Total Solids.—The amount of the total solids of the urine is most exactly determined by accurately weighing the residue obtained after complete evaporation of the water from a definite volume of urine. For practical clinical purposes, however, the total solids may be calculated with approximate correctness from the specific gravity, which necessarily bears a definite ratio to the proportion of solids in solution. To make this calculation, multiply the last two figures of the specific gravity as ordinarily expressed by .233 (.2 according to some authorities); the product gives the *percentage* weight of solids present. The total solids in twenty-four hours can then be calculated from the daily amount of urine.

Urea.—The quantitative estimation of urea is one of the more important procedures of urine analysis. Urea is determined from the volume of nitrogen given off in the decomposition of urea by the action of sodium hypobromite (or sodium hypochlorite) with an excess of sodium hydrate. The reaction which takes place is about as follows:



The purpose of the NaOH is to take up the CO₂ derived from breaking up of the urea, leaving nitrogen as the only free gas evolved, from the volume of which the amount of urea can be calculated.

Since the molecular weight of urea is 59.95, of which 28.02 is nitrogen, the weight of the urea is 2.1895 times that of the nitrogen evolved. The weight of 1 cubic centimetre of nitrogen at a temperature of 0° C. and a barometric pressure of 760 millimetres of mercury is .001255 gram; therefore each cubic centimetre of nitrogen evolved in the test at 0° C. and a net pressure (after deducting tension of watery vapor) of 760 mm. mercury theoretically represents .002685 gram of urea. According to numerous observers, however, the total amount of nitrogen theoretically required is not actually given off in the test; but the different observers are not agreed as to the precise deficiency, the deficit found ranging from zero to 8 per cent. Hüfner's results, that urea yields only about 95 per cent of its nitrogen in the test, are adopted by some urine analysts as a basis; according to this, 1 cubic centimetre of nitrogen (0° C., 760 mm.) would represent .00283 gram of urea. In urine analysis, uric acid, proteids, and other nitrogenous substances yield a small amount of nitrogen with the hypobromite method, and as this may be assumed to offset the deficit from urea, the coefficient required by theory may be accepted in practice.

In practice, for the most accurate results the temperature, barometric pressure, and tension of aqueous vapor must be taken into account in calculating the amount of nitrogen and of urea. While in localities near the sea-level slight variations of the barometric pressure may perhaps be ordinarily disregarded, in high altitudes it must be taken into account or a very large error (up to 100 per cent of the true value in the highest inhabited regions) will be added. Assuming that all the nitrogen is given off by the urea, and that the aqueous vapor within the gasometric tube is saturated, the amount of urea in the portion of urine tested may be determined by the following general formula, in which

U = the weight of urea, in grams, in the urine tested;

N = the volume, in cubic centimetres, of nitrogen actually observed;

T = the temperature, Centigrade, at which the observation of the volume of nitrogen is made;

B = the barometric pressure at the time of observation, in millimetres of mercury;

A = the tension of aqueous vapor at the temperature T:

$$U = \frac{.002685 N (B - A)}{760 (1 + .008666 T)}$$

This formula may be simplified as follows:

$$U = \frac{.000003533 N (B - A)}{1 + .008666 T}, \text{ or}$$

$$U = .000003533 N (B - A) \left(\frac{1}{1 + .008666 T} \right).$$

The maximum tension of aqueous vapor in millimetres of mercury, and the value of $\frac{1}{1 + .008666 T}$ at various temperatures, are as follows:

Temperature.		Maximum tension of aqueous vapor.	Value of $\frac{1}{1 + .00366 T}$.	Temperature.		Maximum tension of aqueous vapor.	Value of $\frac{1}{1 + .00366 T}$.
C.	F.			C.	F.		
0°	32.0°	4.6	1.0000	25°	77.0°	23.5	0.9160
10	50.0	9.2	.9646	26	78.8	25.0	.9130
11	51.8	9.8	.9612	27	80.6	26.5	.9099
12	53.6	10.5	.9579	28	82.4	28.1	.9069
13	55.4	11.2	.9545	29	84.2	29.8	.9039
14	57.2	11.9	.9512	30	86.0	31.5	.9009
15	59.0	12.7	.9479	31	87.8	33.4	.8979
16	60.8	13.5	.9446	32	89.6	35.3	.8950
17	62.6	14.4	.9413	33	91.4	37.4	.8921
18	64.4	15.4	.9381	34	93.2	39.5	.8892
19	66.2	16.3	.9349	35	95.0	41.8	.8863
20	68.0	17.4	.9317	36	96.8	44.2	.8834
21	69.8	18.5	.9285	37	98.6	46.7	.8805
22	71.6	19.7	.9254	38	100.4	49.3	.8777
23	73.4	20.9	.9223	39	102.2	52.0	.8749
24	75.2	22.2	.9191	40	104.0	54.9	.8721

At various temperatures, under a barometric pressure of 760 mm. of mercury and allowing for water-vapor tension, the weight of urea corresponding to each cubic centimetre of nitrogen evolved in the hypobromite test is as follows:

Temperature.		Weight, in grams, of urea corresponding to 1 c.c. of nitrogen.	Temperature.		Weight, in grams, of urea corresponding to 1 c.c. of nitrogen.
C.	F.		C.	F.	
16°	60.8°	.00249	28°	82.4°	.00234
18	64.4	.00247	30	86.0	.00232
20	68.0	.00244	32	89.6	.00229
22	71.6	.00242	34	93.2	.00226
24	75.2	.00240	36	96.8	.00223
26	78.8	.00237			

At ordinary temperatures and pressure, the coefficient is about .0025 for each cubic centimetre of nitrogen. Ordinarily, therefore, the percentage of urea can be quickly and approximately calculated by dividing the number of cubic centimetres of gas by 4 (1 c.c. of urine having been used for the test).

In brief, the volume of the nitrogen evolved being determined by the methods given below, the corresponding weight and percentage of urea may be calculated by one of the following methods:

(a) For a simple and approximate mode of calculation, divide the volume of nitrogen evolved from 1 cubic centimetre of urine, expressed in cubic centimetres, by 4; the quotient is the *percentage* of urea, by weight.

(b) Where the barometric pressure is near 760 millimetres of mercury, so near that slight variations can be disregarded, multiply the volume of nitrogen obtained, in cubic centimetres, by the coefficient for the temperature at which the observation was made, as given in the table on page 186; the result expresses the weight, in grams, of the urea in the volume of urine used in the test; from which the percentage can be readily calculated.

(c) If the most accurate results are desired, calculate by the formula

$$U = .000003533 N (B - A) \left(\frac{1}{1 + .003666 T} \right).$$

That is, from the barometric pressure at the time of the test subtract the tension of aqueous vapor at the given room temperature (see table on page 186); multiply together this result, the coefficient .000003533, the volume of nitrogen evolved in the test, and the value of $\frac{1}{1 + .003666 T}$ at the temperature of the test (see table on page 186). The result gives the weight of urea, in grams, in the portion of urine tested; from which the weight percentage can be calculated.

For example, if 10 cubic centimetres of gas is evolved from 1 cubic centimetre of urine, at a room temperature of 20° C. and barometric pressure of 750 millimetres of mercury, the corresponding amount of urea calculated by the three methods would be 2.5, 2.44, and 2.41 per cent respectively.

Another method that may at times be useful is to make a control test with a 2-per-cent solution of urea, and calculate the urea of the urine from the volume of gas evolved from the urea solution of known strength.

The hypobromite test solution employed in the urea test must be prepared fresh each time before use, as follows: To 10 or 15 parts by measure of a 40-per-cent stock solution of sodium hydrate add 1 part of bromine; mix thoroughly and then add an equal volume of water. To minimize the irritating fumes, the stock of bromine in its bottle should be kept covered with a layer of water, and the proper amount of bromine removed with a pipette.

To carry out the test special arrangements of apparatus are necessary for decomposing the urea and measuring the nitrogen generated. Numerous forms of apparatus have been devised for

this purpose, all more or less efficient and convenient, of which the following may be commended:

1. *Doremus's ureometer* (Fig. 31) consists of a graduated closed tube with an overflow bulb; it is accompanied by a pipette with a mark representing 1 cubic centimetre. The hypobromite fluid is poured into the bulb, and the instrument tilted so that it runs into and fills the long arm. Sufficient fluid should be used so that when held upright the tube is entirely full, with enough fluid in the bulb to prevent the access of air to the tube. The urine to be tested is then drawn into the pipette to the 1 c.c. mark. The curved point of the pipette is passed to the bottom of the long arm of the ureometer and the



FIG. 31. — Doremus's Ureometer, with Support. (Lentz & Sons.)

urine slowly expelled into this arm by compressing the rubber bulb. Brisk effervescence occurs, and the evolved nitrogen rises to the top of the tube, where, after allowing a sufficient time for the completion of the reaction, the equalization of the temperature, and the subsidence of the froth, the gas is read from the graduation. The instrument is graduated to show directly either fractions of a gram of urea in each cubic centimetre of urine, or grains of urea to the ounce of urine; from this, if desired, the percentage can be easily calculated. The apparatus has a range up to 3 per cent. Urine containing over 3 per cent of urea should be diluted with an equal amount of water, the mixture tested, and the results obtained multiplied by 2. With albuminous urine the froth subsides very slowly.

This apparatus gives results sufficiently reliable for ordinary clinical purposes, although not precise enough for very exact determinations. A certain amount of gas usually escapes while the urine is being discharged from the pipette, which interferes with great accuracy. It should be noted that the 1 c.c. mark on the pipette is relative only to the instrument which it accompanies, and does not necessarily indicate exactly 1 cubic centimetre; hence with each ureometer only the particular pipette pertaining to it (or one of like volume) should be used.

2. *Author's Ureometer* (Fig. 32).—The author has had an apparatus constructed for the estimation of urea which is very easy to manipulate and accurate in its results. It consists of a graduated closed tube *c*, an overflow bulb *b*, and an open arm *a*. Projecting obliquely downward from the tube *c* is a side arm *d*, which is closed off from the lumen of the main tube by a glass stopcock *e* containing a perforation *n*. The capacity of *d* and *n* together is exactly 1 cubic centimetre. To make the test, the glass stopper is turned so that its perforation *n* opens communication between the side tube *d* and the main tube *c*. A small amount of the urine is poured into the open arm *a*, and the instrument tilted so that the urine fills *d* and *n*; this urine, which is introduced for the purpose of rinsing out the tube *d*, is then drained away. When sufficiently rinsed, a portion of the urine is introduced into the side arm, completely filling it and the perforation in the stopper; the stopper is then turned so as to close off the side arm from the main tube. In this manner, exactly 1 cubic centimetre of urine is segregated in the spaces *d* and *n*, which should be entirely free of air bubbles. The main tube is then rinsed out with water, and sufficient hypobromite solution introduced to fill the graduated arm *c* completely when in an upright position. The stopper *e* is then turned so as to open communication between arms *d* and *c*, and allow the urine to be acted on by the test fluid; the rapidity of the reaction can be controlled with the stopcock. Effervescence occurs, and the nitrogen evolved rises and fills the upper end of the graduated

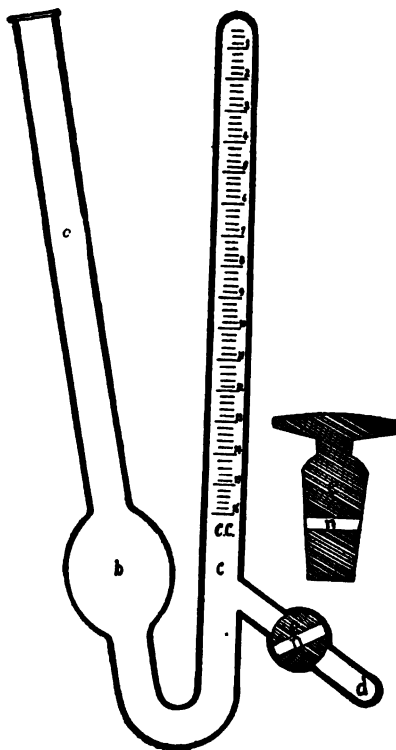


FIG. 32.—Author's Ureometer.

arm. When the nitrogen is completely evolved and the temperature of the gas and liquid has subsided to that of the room, water is poured into the open tube *a* until the level of the fluid in the two arms is the same, thus equalizing the hydrostatic tension on the gas. The volume of gas is then read off, in cubic centimetres and tenths; from which and the data as to temperature and barometric pressure the percentage of urea can be calculated in the manner

given above. The graduation covers a range up to 4 per cent of urea. If desired, the graduation could be made to show the approximate percentage of urea at ordinary room temperature and sea-level atmospheric pressure.

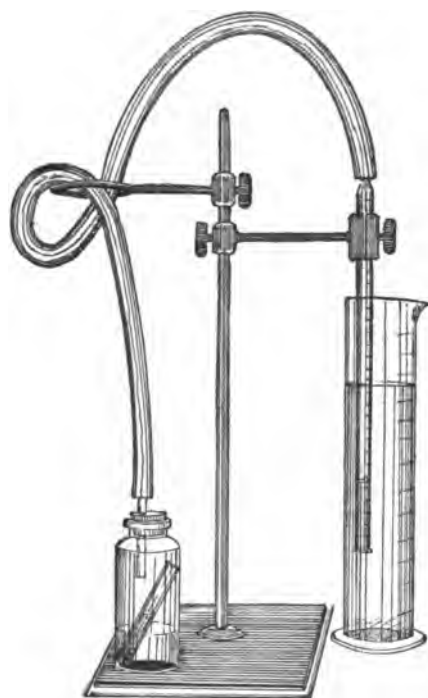


FIG. 33.—Apparatus for Estimation of Urea.

3. A very efficient arrangement for estimating urea can be improvised from ordinary laboratory apparatus (Fig. 33). The test solution is placed in a wide-mouthed bottle. A measured amount of urine (1 or 2 cubic centimetres) is put into a slender cylindrical glass receptacle of suitable size, which is then set upright inside the glass bottle; this keeps the two fluids separate until the time arrives to mix them. The bottle is closed by a stopper penetrated by a glass tube, which is connected by a long rubber tube with a burette clamped in an inverted position with its major portion in a tall, cylindrical glass vessel of water. After arranging the test fluid and urine in the bottle, inserting the stopper tightly, and waiting until the temperature becomes equalized, the burette is moved up or down until the water within it is at the same level as that without; the graduation on

the burette corresponding to this level is noted. The bottle is then tilted so that the urine in the container inside mixes with the hypobromite solution. The water inside the burette is depressed to an extent equal to the volume of nitrogen evolved. After waiting ten or fifteen minutes or until the temperature is equalized, the burette is raised until the water inside it is on a level with the water outside, when the corresponding graduation is again read. The difference between the two readings gives the volume of nitrogen generated, from which the urea can be calculated.

4. *Squibb's apparatus* can also be readily improvised. The arrangement for mixing the urine and test fluid is the same as in that just described. The volume of nitrogen is determined from the volume of water displaced by the gas generated.

Uric Acid.—Deposits of uric acid or urates are ordinarily easily recognizable by their microscopical appearance and their solubility with alkalis or heat. Occasionally, chiefly in connection with calculi, chemical qualitative tests are required to determine if suspected material contains uric acid. For this purpose the following tests may be used:

Murexide Test: In a porcelain dish dissolve a small portion of the dry material or residue from evaporation in two or three drops of nitric acid; evaporate by gentle heat; when cool add a drop or two of aqua ammoniæ, when if uric acid or urates are present a spreading purple-red color appears. Some of the xanthin bases give a similar reaction; but on the addition of a drop of sodium-hydrate solution, if uric acid is present, the color changes to a reddish blue and disappears with heat.

Silver Test: Place a drop or two of a solution of silver nitrate on filter paper, and add a like amount of sodium-carbonate solution in which the suspected material has been dissolved; the formation of a black or grayish color indicates the presence of uric acid. This test is said to be very delicate.

Quantitative Estimation: A number of methods of determining the amount of uric acid have been presented, all quite complicated and none closely accurate. One of the easiest and most satisfactory of these is the following modification of the Hopkins method:

In 50 or 100 cubic centimetres of the urine, accurately measured, dissolve 5 or 10 grams, respectively, of powdered ammo-

nium sulphate, and add sufficient ammonia to make the fluid barely alkaline. Allow the fluid to stand two or more hours, with occasional shaking, when the uric acid will be all precipitated as ammonium urate. Filter through a small, thin filter-paper, collecting the precipitated urate on the filter. Then thoroughly wash the filter-paper and precipitate free from chlorides, etc., by passing a 10-per-cent solution of ammonium sulphate through it several times. Break a hole through the bottom of the filter with a glass rod, and with a pipette or wash-bottle blow 100 cubic centimetres of hot water in forcible jets upon the filter paper so as to wash the precipitated urate entirely from it into a beaker or wide-mouthed bottle beneath. Add 15 cubic centimetres of strong sulphuric acid to the fluid containing the precipitate, and titrate the mixture immediately, while hot, with one-twentieth normal solution of potassium permanganate. Until the uric acid is completely saturated the permanganate is instantly decolorized on being added to the titrated fluid; the titration should be continued until the fluid being tested, thoroughly mixed with the permanganate, remains of a pale-pink color throughout for a few seconds. This is not a very sharp end reaction, but it is sufficient to obtain practical results. Then multiply the number of cubic centimetres of the permanganate solution employed in the titration by .00375; the product expresses the weight, in grams, of the uric acid present in the amount of urine taken for the test.

The twentieth-normal test solution is prepared by dissolving exactly 1.577 grams of pure dry potassium-permanganate crystals in 1 litre of water. It should not at any time be allowed to come in contact with organic material; it should therefore be kept in glass-stoppered bottles, and used in a burette controlled by a glass stopcock, without a rubber tube attached.

Indican.—The amount of this substance can be estimated roughly, but with sufficient accuracy for clinical purposes, by the following simple test of Heller: With 4 cubic centimetres of strong hydrochloric acid in a porcelain capsule or a test tube thoroughly mix 5 to 20 drops of urine. With the amount of indican normally present a characteristic pink color appears. If indican is in excess a violet or blue color develops immediately or within three or four minutes; the greater the intensity or rapidity with which the blue color develops, and the smaller the

amount of urine that produces it, the greater is the amount of indican. If, on the contrary, the pink color is produced tardily and faintly, indican is diminished; and if no pink tinge appears, especially after adding a drop or two of nitric acid or other oxidizing substance, indican is practically absent.

If bilirubin is present or the urine is highly colored, the addition of lead acetate will decolorize the urine and make the indican test more practicable.

Phosphates.—All the phosphates of the urine, earthy and alkaline, are precipitated together by adding about one-third of its volume of "magnesian fluid," which consists of magnesium sulphate 1 part, ammonium chloride 1 part, aqua ammoniæ 1 part, water 8 parts. The precipitate consists of a mixture of earthy and ammonio-magnesian phosphates. The total amount of this precipitate can be determined gravimetrically by adding filtered magnesian fluid to a measured amount of clear urine (50 or 100 cubic centimetres), and then collecting the precipitate on a filter-paper previously exactly weighed after being well dried in a hot oven. The precipitate and filter-paper are then repeatedly and thoroughly washed with an abundance of pure water made markedly alkaline with ammonia, to remove all other substances. The filter-paper and precipitate are then thoroughly dried in a hot oven as before, and carefully weighed. The increase in weight indicates the weight of the precipitated phosphates in the volume of urine employed for the test.

The *earthy phosphates* when present undissolved in urine are recognizable from the microscopical appearance and their prompt solubility on adding a few drops of any acid. They can be completely precipitated by themselves from urine by making it strongly alkaline with sodium, potassium, or ammonium hydrate, and then heating. Thus precipitated from a measured amount of clear urine (as 100 cubic centimetres), their quantity can be determined gravimetrically by collecting them on a weighed filter, washing thoroughly with ammoniacal water, drying and weighing again, in the manner just described for the total phosphates.

The *alkaline phosphates* can be precipitated separately, in the form of ammonio-magnesian phosphate, by adding magnesian fluid to the filtrate obtained after precipitating and removing the earthy phosphates as above. The precipitate thus obtained from

a definite amount of urine can be collected on a filter and weighed in the same manner as just described; the result expresses the weight of *triple phosphate* formed from the sodium and potassium phosphates.

These gravimetric methods give results sufficiently useful for approximate comparative purposes, but as the precipitate consists of a mixture of different phosphates, the amount of contained phosphoric acid cannot be exactly determined.

Volumetric Estimation of Phosphoric Acid.—The amount of phosphoric acid, calculated as P_2O_5 , may be estimated volumetrically. The solutions required are as follows:

a. A standardizing solution of 10.085 grams of Na_2HPO_4 in 1 litre of water. The phosphate must be weighed in the form of dry, well-formed crystals, which have not lost any of their water of crystallization. This solution contains 0.2 per cent of P_2O_5 .

b. A solution containing 35.511 grams of pure and well-formed crystals of uranic nitrate, $UO_2(NO_3)_2 + 6H_2O$, in a litre of water. This may be prepared as nearly as possible by weight, or slightly stronger than desired, and then brought to the standard after titrating it with 50 cubic centimetres of the foregoing solution of sodium phosphate in the same manner, described below, as urine is titrated. Twenty cubic centimetres of the uranic solution should be equivalent to 50 cubic centimetres of the phosphate solution. Each cubic centimetre of the uranic solution therefore represents .005 gram of P_2O_5 .

c. Sodium-acetate solution, consisting of sodium acetate 10 parts, glacial acetic acid 5 parts, water sufficient to make 100.

d. Saturated potassium-ferrocyanide solution, freshly prepared.

To make the estimation, add 5 cubic centimetres of the sodium-acetate solution to 50 cubic centimetres of urine, and heat in a water bath to 90° or 100° C. Titrate, while thus heated, with the uranic-nitrate solution. A precipitate of uranic phosphate falls until the urinary phosphates are exhausted, after which the excess of uranic nitrate will give a reddish-brown precipitate with potassium ferrocyanide. From time to time during the titration, transfer a drop of the urine, well stirred, to a porcelain dish and touch it with a drop of the potassium-ferrocyanide solution; as soon as this shows a reddish-brown color, discontinue the titration. Multiply the number of cubic centi-

metres of the uranic-nitrate solution required to saturate the urinary phosphates by .005; the product expresses the weight, in grams, of P_2O_5 in the 50 cubic centimetres of urine tested. It may be found convenient to make two titrations each time, the first to determine the approximate result, the second to arrive at an exact figure.

To estimate the P_2O_5 of the earthy phosphates alone by this method, precipitate them by alkali and heat, collect them on a filter and wash with ammoniacal water, as above indicated; then break a hole in the bottom of the filter, wash the precipitate from the paper with 50 cubic centimetres of water, in jets, and dissolve it by adding as little acetic acid as is necessary. Add 5 cubic centimetres of the sodium-acetate solution and titrate while hot with uranic-nitrate solution in the manner described.

The P_2O_5 of the alkaline phosphates may be determined by subtracting the amount found combined with earthy bases from the total amount found; or by titrating the urinary filtrate after removing the earthy phosphates.

Carbonates.—The presence of carbonates is shown by the occurrence of brisk effervescence upon adding acetic or other acids. In the nitric-acid contact test for albumin, bubbles of nitrogen slowly collect on the sides of the test-tube from decomposition of urea; this is much slower than the effervescence of carbonates and does not occur with other acids than nitroso-nitric.

Albumin.—Many tests for albumin in the urine have been introduced, some too delicate, some not sensitive enough, most of them not needed. Only two tests need ordinarily be used, which have been in use for many years, answer all requirements as to sensitiveness and facility, and permit quantitative estimations sufficiently approximate for practical comparative purposes; these are the nitric-acid contact test and the heat test.

For applying these tests satisfactorily it is important that the urine be perfectly clear. Large amounts of albumin are easily shown even if the urine be turbid; but small amounts can be demonstrated with certainty only with clear urine. Fresh urine not contaminated with bacteria can be clarified by filtration. If bacteria or very fine particles are abundant the turbidity due to them cannot be removed by filter-paper. In such cases filtration after shaking the urine with insoluble powders like talc, etc.,

may clear it; but this procedure also removes a portion or all of the albumin present, and hence is likely to lead to error. Vigorous centrifugation may clear urine sufficiently for the test. Filtration through Chamberland's filter will completely clarify urine.

By passage through filter paper urine or pure water may take up vegetable albumin, but under ordinary circumstances only in amount so minute as not to react with the tests here described or with any but the most extremely sensitive tests for albumin.

Nitric-acid Contact Test (Heller's test).—This test is performed by placing a small amount of strong nitric acid in a test tube or conical glass, and then carefully overlaying it (see page 10) with the urine to be tested, which should be clear. If albumin is present, a sharply defined white opaque layer or disc several millimetres in thickness appears at the junction of the two fluids. If only a minute trace of albumin is present there may be only a faint cloudiness at the contact zone, instead of a sharp white disc. In this test, not only serum-albumin, but also acid or alkali albumin, globulin, and albumose are precipitated. The reaction is best observed against a dark, unlighted background, the tube itself being well lighted. The reaction is sometimes slow in developing; hence the fluids should be observed for fifteen to thirty minutes before a negative conclusion is arrived at. The sensitiveness of the test is probably increased by using hot urine, or by warming the test-tube containing the fluids in hot water; heat thus applied clears away any troublesome cloud of urates. Sometimes the albuminous disc may be more or less colored by urinary pigments.

This test is very sensitive to albumin; and while other tests are more delicate, they are also untrustworthy, and the contact test is sufficiently sensitive for all clinical purposes. It affords little reliable information, even approximate, as to the amount of albumin present. The method is slightly more sensitive with alkali-albumin than with acid-albumin, though under the conditions ordinarily presented by the urine the difference is scarcely appreciable.

This contact test presents a number of other features with which the urine examiner should be familiar.

Nucleo-albumin or mucin, if abundant, may be precipitated in a *diffuse* cloud in the urine just *above* the contact zone. It is

important not to mistake this for albumin, which forms usually a *sharply defined* white disc at the contact plane. Still, great difficulty may be found at times in distinguishing a mucoid from a faint albuminous reaction.

After the ingestion of certain resinous substances, as copaiba or turpentine, the urine when tested by the nitric-acid contact method yields a white disc closely resembling that of albumin. This resinous precipitate is redissolved on the addition of alcohol, wherein it differs from the albuminous precipitate.

If the urates are concentrated in the urine, the nitric-acid contact method may cause them to be gradually precipitated in the form of a faint cloud in the upper part of or throughout the urine. An excessive or rapidly produced cloudiness of this kind may roughly indicate an excess of uric acid. If a cloud of urates interferes with the albumin test, it may be obviated by introducing the urine hot, or by warming the test-tube in hot water or, carefully, over a flame; or the urates may be kept in solution by adding alkali to the urine.

With most urines in the performance of this test numerous bubbles of gas slowly form at the contact zone, and rise to the surface or adhere to the sides of the glass. These are caused by the action of traces of nitrous acid on the urea and uric acid, which are decomposed with the evolution of nitrogen and carbon dioxide. This effervescent action is most marked when impure nitric acid of a yellow tinge and containing considerable nitrous acid is used; but even pure colorless nitric acid causes a certain amount of bubble formation.

If carbonates are present, brisk effervescence from that source occurs.

If the urine is cloudy from phosphates, it clears in the vicinity of the acid. When the urine contains a large proportion of urea (4 or 5 per cent or more), the contact-test is apt to cause a white crystalline disc of urea nitrate to form slowly at the junction of the fluids. This can hardly be mistaken for albumin, since its crystalline structure is easily recognizable, and on shaking it up with the urine or diluting with water it redissolves.

Nitric acid applied by the contact method produces marked color reactions with the pigments and chromogens of normal and abnormal urines. In normal urine a diffuse pink color forms at

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the junction of the fluids, fading away above; this seems partly at least to be due to the action of the acid on indican. In certain abnormal urines, as when concentrated or in febrile conditions, the color reactions are strongly marked, a darker color layer appearing, sometimes reddish, often a deep brown, diffuse or sharply defined; this may be partly due to indican, partly, probably, to excessive or abnormal pigments. If bilirubin is present, its characteristic series of colors, beginning with green, appears at the contact plane; this test therefore serves to show the presence of both albumin and bilirubin. If iodides are present in the urine the liberation of iodine causes the formation of a brown layer.

Heat Test.—This test consists in boiling the urine to coagulate any albumin present, followed by the addition of nitric acid to increase the proteid reaction and eliminate other reactions. A column of clear urine 5 to 10 centimetres in depth is boiled in a test-tube; first the upper part of the fluid may be boiled, in order to detect any cloudiness that may appear by comparison with the lower part. Later the entire urine may be boiled to precipitate all the albumin. The development of a cloudiness or precipitate on heating will be due to precipitation of either earthy phosphates or albumin. A drop or two of nitric acid is then added; if the turbidity is due to phosphates it will immediately clear up, while if due to albumin it will remain or increase. Whether a cloudiness appears or not, nitric acid is added, two or three drops at a time, until a precipitate forms or ten or fifteen drops have been added. Sometimes the albumin is not precipitated until several drops of the acid are added. A precipitate appearing after the urine cools, redissolving on again heating, is uric acid or albumose. By the combined action of heat and nitric acid, albumin is precipitated in fine granules or feathery flakes, sometimes white, sometimes colored by urinary pigments.

As a qualitative test, this method is rather less delicate than the contact test, as it may fail to react when the latter gives decided response. It is useful as an approximate quantitative test, sufficient for comparative purposes. For this purpose the boiled and acidulated urine is set aside upright in a test-tube, or better a graduated tube, and the precipitate, which should be broken up into granular portions by shaking, allowed to settle for twelve to twenty-four hours, or thrown down immediately with

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the centrifuge. When the precipitate is compactly sedimented in the bottom of the tube, its relative volume compared with the volume of the entire urine (as $\frac{1}{4}$, $\frac{1}{8}$, etc.) is noted. It should be observed that this is the amount by *bulk* and not by *weight*; the weight percentage of albumin in urine is always comparatively small (rarely over two and usually under one per cent), while the corresponding amount by relative bulk appears large and conspicuous. The bulk ratio is but a rough quantitative method, but is sufficient for comparison of the progress of the case.

For more accurate quantitative measurement of the albumin precipitated by heat and acid, it may be collected on a weighed filter, thoroughly washed, dried, and weighed; by deducting the weight of the filter paper, the amount of albumin is obtained.

In practical work, it is convenient to apply the contact method first as a qualitative test, and then if this yields a positive result to employ the heat test for quantitative purposes.

The precipitate thrown down by this method contains serum-albumin, globulin, and when cool albumose.

Nucleo-albumin, or mucin, likewise forms a diffuse cloud with heat and acid, and must be carefully distinguished from albumin. If the cloudiness be due to mucin, a similar cloud can be produced in unboiled urine by the addition of acetic acid, which does not so react on albumin. In doubtful cases, sodium chloride may be added to the urine to hold the mucin in solution, and the boiling and acid method tried, when a positive result will indicate albumin.

Resinous drugs may cause a cloudiness of the urine in this test as in the contact test; the precipitate is re-dissolved with alcohol. Urates are not thrown down in the hot urine. An evolution of gas bubbles from decomposition of urea by nitrosotric acid occurs as in the contact test. Decided color reactions similar to those of the contact test take place on adding nitric acid to hot urine, pink or darker colors being produced throughout the fluid; or a bright green develops, followed by other colors, if bilirubin is present.

If it is desired for any purpose to remove albumin from the urine coagulate it well by boiling, and then filter.

Nucleo-albumin, or mucin, is detected chiefly by its being precipitated on the addition of an excess of acetic acid. It is some-

times advisable to dilute the urine before testing, to prevent precipitation of uric acid, and by diluting the chlorides of the urine to diminish the solubility of the mucin. The importance of distinguishing between mucin and a faint albuminous reaction has been considered above.

Sugar.—Numerous tests, qualitative and quantitative, are available for the sugars, especially glucose, chiefly based on their reducing power over copper or bismuth salts, their property of undergoing alcoholic fermentation with yeast, their action on polarized light, and their reaction with phenylhydrazin. Occasionally a combination of tests is necessary to differentiate the different kinds of sugar from one another or from other reducing substances.

Copper Test.—This test depends on the power possessed by glucose and other sugars of reducing cupric oxide, CuO , to cuprous oxide, Cu_2O . This reaction is yielded alike by glucose, levulose, lactose, maltose, pentoses, glycuronic acid, alkapton, and certain adventitious substances derived from ingesta. The cupric oxide is prepared by mixing copper sulphate with an excess of caustic alkali, with some organic substance to make the cupric oxide thus formed soluble in the alkali. The test is capable of both qualitative and quantitative application. Several formulæ for the test have been introduced, as Fehling's, Pavy's, Trommer's, etc.

The *glycero-cupric test* may be recommended as probably the best of the copper tests for glucose. For qualitative purposes the solution may be prepared as follows: Dissolve 4 grams of pure copper sulphate in 30 cubic centimetres of water, and add 30 cubic centimetres of pure glycerin. When the test is to be made mix about 1 cubic centimetre of this solution with 5 cubic centimetres of liquor potassæ or sodæ (about 5-per-cent solution of potassium or sodium hydrate). Boil the test fluid thus freshly prepared for a few seconds, to determine if the solution itself is good, in which case it will undergo no change. Then add two or three drops of the urine to be tested, and boil again for fifteen to thirty seconds (not too long); if no reaction appears, add two or three more drops of urine and boil again; continue thus until a reaction develops, or until two or three cubic centimetres of urine are added. Sometimes the reaction does not develop until after several minutes. If glucose or other reducing substances

are present in material amount, the blue color of the test fluid changes to a yellow or orange, and a copious orange or red precipitate is thrown down; this reaction should appear promptly after the addition of 2 to 10 drops of the urine. The more abundant the sugar the less is the amount of urine required to produce a vigorous reaction. If the glucose or other reducing substance is in minute amount, it will require a larger quantity of urine (over 10 drops) to give a reaction; which in this case will consist simply in a change of color of the test fluid to amber or green, without a precipitate. So slight a reaction as this indicates only a minute amount of glucose or some other reducing agent besides glucose, and can be fairly disregarded clinically as being within a normal limit. The test should always be begun with a very few drops of urine.

During the procedure the earthy phosphates are always precipitated by the alkali and heat, in a whitish or grayish cloud, which should not be (but sometimes is) mistaken for a sugar reaction. Albumin, if present, may give the biuret reaction in this test, turning the fluid a violet color; albumin does not often interfere with the operation of the sugar reaction, but if it is suspected of so doing or is in excessive amount it may first be removed.

As a qualitative test this method is adequately sensitive, but has the disadvantage of reacting with other substances (which are, however, rarely present or sufficient to give a vigorous reaction) besides glucose.

The glycerocupric and other copper methods may be employed for quantitative glucose tests, the sugar being computed from the amount of urine required exactly to decolorize a solution containing a known amount of copper salt. The method has not proven very satisfactory, partly from the instability of the solutions, partly from the indefiniteness of the end reaction. For ordinary purposes the fermentation method is preferable.

Purdy's Method, which is typical of and probably as good as any of the quantitative copper tests, is as follows: Dissolve 4.742 grams of pure copper-sulphate crystals and 88 c.c. of pure glycerin in 200 c.c. of pure water, with gentle heat; dissolve 28.5 grams of pure potassium hydrate in another 200 c.c. of water; mix the two solutions; when cooled add 450 c.c. of pure aqua ammoniæ fortior (U. S. P., specific gravity .9), and dilute the whole with water to exactly 1 litre. Thirty-five cubic centimetres of this standard solution is exactly reduced and decolorized while boiling by .02 gram of glucose. Place exactly 35 c.c. of the solution in a sufficiently large flask, dilute with about twice as much pure water, and bring thoroughly to the boiling-point. From

a burette above, the urine to be tested is discharged drop by drop into the boiling test fluid until the latter is just completely decolorized. The amount of urine required for this result contains exactly .02 gram of glucose; from which the percentage can be calculated. If the urine contains much sugar it is best to dilute it accurately to a known degree, and, after titrating with it thus diluted, to make the proper allowance.

Fermentation Test.—Under the action of yeast fungi, glucose, levulose, and maltose undergo alcoholic fermentation, breaking up into alcohol and carbon dioxide. This affords a useful qualitative test for differentiating glucose from all other reducing substances except levulose and maltose, and the most practicable quantitative method of estimating glucose in the urine.

For *qualitative* purposes the test is best applied by means of a "fermentation tube" (Fig. 41), consisting of an upright glass tube closed above and opening into an overflow bulb below (similar to Doremus's ureometer, which can be used for the purpose). Sufficient urine to fill the tube is well mixed with one or two grams of fresh yeast, and then introduced into the tube so that in the upright position the tube is completely filled with urine, with no air at the top. The tube is then set aside for eighteen to twenty-four hours at a temperature of 30° or 35° C. to allow fermentation to take place. If glucose is present it is decomposed, and the carbon dioxide copiously evolved rises in the tube, progressively depressing the level of the fluid. A small bubble of gas ordinarily appears at the top of the tube even in normal urine; but the accumulation of more than such a bubble, as shown, if needed, by comparison with a control test with normal urine, indicates the presence of a fermentable sugar, ordinarily glucose. Antiseptics should not have been added to the urine for preservative purposes, or the action of the yeast will be inhibited. In default of a fermentation tube a test-tube can be completely filled with the yeast-urine mixture and inverted, still filled, in an open glass containing urine. As .004 gram of glucose yields 1 cubic centimetre of CO₂ (at 0° C. and 760 millimetres pressure) it does not require a very large proportion of glucose completely to fill the tube with gas, and the test is quite delicate.

For *quantitative* purposes the differential specific-gravity method is the best, the fermentation tube not affording satisfactory results. The principle is this, that the loss of CO₂ causes a

decrease of the specific gravity of the urine, and from this decrease the amount of CO_2 and of glucose can be calculated. In practice, after taking the specific gravity 75 or 100 cubic centimetres of urine (not containing antiseptics) is mixed with two or four grams of yeast and set aside in a warm place, at about 30° or 35°C. , in a bottle with a hole through or a notch cut alongside the cork to permit escape of air. Fermentation is usually complete after twelve to twenty-four hours; its conclusion will be shown by a negative response to the copper test. When fermentation is complete the specific gravity of the fermented urine is again taken, at the same temperature at which it was taken before fermentation. The difference between the two, multiplied by .23, gives approximately the percentage of glucose by weight. Thus, if the specific gravity was 1.036 before and 1.007 after fermentation, the difference, 29, multiplied by .23, indicates that there is $6\frac{2}{3}$ per cent by weight of glucose present. The method involves a delay of twenty-four hours, and is only approximate in its results, but it is easy to carry out and is sufficiently accurate for ordinary clinical and comparative purposes.

In routine urinary work, sugar can be conveniently tested for qualitatively by the glycerocupric method, and if this shows the presence of sugar, its amount can then be determined by the specific-gravity fermentation method.

Polariscopic Tests.—The differentiation between different kinds of sugars and other reducing substances is aided by tests of their action on polarized light, as to the existence, direction, and amount of their rotatory action (see page 158). This method also affords an approximate means of quantitative estimation of sugar.

Phenylhydrazin Test.—Glucose, levulose, maltose, some pentoses, and glycuronic acid when treated in a certain way with phenylhydrazin yield characteristic microscopic osazone crystals, which differ from one another somewhat in appearance and melting-points. Some authorities commend the method as delicate and reliable; but as originally practised it is a rather troublesome process and does not appear to offer any advantages over the methods given above as to sensitiveness, or as to ability to differentiate between the various reducing substances.

Acetone.—Several tests for acetone have been presented, aside from its odor, among which the following qualitative methods may be mentioned:

Legal's Test: Make a few cubic centimetres of urine distinctly

alkaline with a little liquor potassæ or sodæ; add a few drops of a rather strong solution of sodium nitro-prusside made just at the time of using. The mixture becomes red, and then rapidly fades. On adding a few drops of strong acetic acid a purple or violet-red color (also transient) develops if acetone is present.

Lieber's Test: Add about 1 per cent of phosphoric acid to the urine and distill 500 or 1,000 cubic centimetres. To a few cubic centimetres of the first 10 to 30 cubic centimetres of the distillate add a few drops of liquor potassæ or sodæ and a few drops of dilute Lugol's solution (page 11). If acetone is present a yellow precipitate of iodoform appears, recognizable by its odor when heated. Alcohol, which might exceptionally occur in the distillate, and lactic acid also give this reaction.

Diacetic acid may be tested for qualitatively as follows: To a few cubic centimetres of fresh urine add drop by drop a rather strong watery solution of ferric chloride; if phosphates are precipitated filter them out and continue adding the ferric chloride. If a Bordeaux-red color appears, boil another portion of the original urine and repeat the test. If no reaction is obtained in the second test, treat a third portion of the urine with sulphuric acid and extract with ether; apply the ferric-chloride test to the ethereal extract. If this extract gives a positive reaction, the color disappearing in twenty-four to forty-eight hours, diacetic acid is present, especially if acetone is abundant.

Beta-oxybutyric acid may be suspected to be present if after removal of sugar by fermentation the urine rotates polarized light to the left. The presence of diacetic and β -oxybutyric acids in large amount is indicated if there is found an excess of ammonia in the urine or a high ratio of ammonia nitrogen (15 per cent or over being of serious significance).

Blood.—The presence of blood (hæmaturia) is shown by the concurrent presence of red corpuscles, albumin, and hæmoglobin; the corpuscles alone are pathognomonic.

Hæmoglobin may be conveniently tested for in the urine by the guaiacum test (page 70). *Heller's test* may also be employed as follows: Add liquor potassæ or sodæ (or if the urine is alkaline and the earthy phosphates are already precipitated add a few drops of magnesian fluid), and heat, so as to precipitate the phosphates; if blood pigment is present the precipitate is colored red. The hæmin test (page 70) may be employed,

being applied to a portion of the dried phosphatic precipitate. The spectroscopic method may also be used, especially for recognizing and differentiating the precise nature of the various derivatives of hæmoglobin (as hæmatoporphyrin).

Pus is recognized by the presence of a large number of leucocytes, with more or less albumin. The sediment may or may not be viscid and tenacious.

Bilirubin may be satisfactorily recognized by the formation of a series of different-colored pigments, beginning with green, (biliverdin), on treatment with an oxidizing reagent. The test is best made with nitroso-nitric acid, that is, impure or so-called "fuming" nitric acid, having a yellow tinge from the presence of nitrous acid. If a sufficiently impure nitric acid is not at hand, "pure" or colorless nitric acid will answer; or a minute portion of copper or mercury may be added to nitric acid to generate nitrous acid; or a fresh mixture of equal parts of nitric and hydrochloric acids may be employed. If the urine is very dark it may be advisable to dilute it. Three to fifteen drops of the acid may be mixed with 5 to 10 cubic centimetres of the urine to be tested; or the urine may be underlaid with the acid. The presence of bilirubin is indicated in the former case by the urine turning a bright or dark green, perhaps subsequently changing to blue and then red. In the contact test, if bilirubin is present a green layer appears, gradually extending upward in the urine; beneath the green are usually blue and red zones.

The test frequently recommended of allowing a few drops each of acid and urine to flow together on a white dish is much less sensitive than the two methods just specified. The development of a green color is absolutely significant of bilirubin, but of green only; no other substance in the urine produces a green color, while other colors than green are produced by various other urinary pigments and chromogens when treated with nitric acid.

In practice the presence of bilirubin is ordinarily sufficiently shown incidentally in the application of the tests for albumin, especially if a yellowish nitric acid is used, so that the same procedure serves for both. If bilirubin is present the characteristic color changes appear at the contact zone in the contact test for albumin, or the albuminous precipitate may be green; and in the heat test, the hot urine readily turns green on the addi-

tion of sufficient nitric acid, or the albumin may be precipitated green.

If altered bilirubin (page 163) is present, the urine from an icteric patient exhibits all the macroscopic characteristics of the presence of bilirubin, but yields no green reaction with nitric acid.

Diazo Reaction.—The application of this test requires three solutions: 1. A saturated solution of sulphanilic acid (para-amido-benzene-sulphonic acid), in a 5-per-cent solution of hydrochloric acid; sulphanilic acid is soluble to the extent of about 2 per cent. Sulphanilic-acid crystals deteriorate so that after being kept for twelve to eighteen months they cannot be relied on to make an effective test solution. 2. A one-half-per-cent solution of sodium nitrite in water; the solution deteriorates, so that it should be freshly prepared every few weeks at least. 3. Aqua ammoniæ.

To make the test, mix 1 part of the sodium-nitrite solution with 40 parts of the sulphanilic-acid solution; add an equal volume of urine and mix; then add about a tenth or a fifth as much ammonia, and mix the fluids. With urine yielding a negative response to the test, the mixture turns a brown or vinegar color; if the diazo reaction is present, the mixture turns a carmine or bright garnet-red color.

Microscopical Examination.—The purpose of microscopical examination is to determine the character of the undissolved solid elements of the urine, or occasionally to examine precipitates artificially produced. The material for the examination, the undissolved solids, may be concentrated and collected either by sedimentation or centrifugation.

To sediment the urine, shake the bottle containing the urine to diffuse the solids thoroughly, and place a quantity of the fluid in a glass receptacle with a conical bottom, adding a few drops of phenol to prevent bacterial growth. Allow it to stand thus for twelve to twenty-four hours, when the solids will have settled to the bottom of the glass and be compacted within a small space. This method is quite efficient, but requires time.

To concentrate the solids by centrifugation, place a portion of the urine, well shaken, in the centrifugal machine and revolve it rapidly. This compacts the sediment densely in the bottom of the tube. This method is very effective, and enables immediate microscopical examination to be made.

For very searching investigations, the two methods may be combined. A large quantity of urine may be sedimented, preferably in a large receptacle with contracted bottom, as a percolator or corked funnel. The undissolved solids of a large volume of urine are thus concentrated at the bottom of the glass. This lower portion of the fluid is then removed by a pipette, or by removing the cork beneath, and further concentrated by the centrifuge; in this manner the sediment from several hundred cubic centimetres of urine can be concentrated within the space of a few drops.

The sediment being concentrated at the bottom of the glass by one of the above-mentioned methods, it is removed for examination by means of a pipette or glass tube, drawn to a slender point at one end. The large end of the tube being tightly closed with the finger-tip, the pointed end is passed to the bottom of the glass containing the sedimented or centrifuged urine, and by carefully and slightly raising the finger-tip the urine and sediment at the bottom are allowed to flow into the pipette till a sufficient amount is collected; the finger is again pressed tightly against the top of the pipette, the tube withdrawn, and a few drops of the urine and sediment are allowed to flow out on a glass slide.

The material thus deposited on the slide may or may not be covered with a cover-glass. If the examination is made with a low-power objective, a cover-glass is not needed, unless the fluid oscillates so as to interfere with observation. If a strong objective is used, a cover-glass is advantageous.

The examination should always be begun with a weak objective (about 2 centimetres), with a strong ocular and the tube lengthened to increase magnification; with this power the sub-stage illumination should be very dim, the iris diaphragm almost completely closed, and the condenser lowered if necessary. An excess of light drowns out most of the faint objects in urine; but they are brought out conspicuously by very dim light. With any power the illumination should be adjusted till the optimum effect is attained. With the low power, epithelium, casts, and most of the urinary objects can be made out perfectly well, or even better than with the high power, while the larger field enables a more rapid and thorough search to be made. The minutest objects, as leucocytes, red blood corpuscles, bacteria, and

calcium-oxalate crystals, are best distinguished with a high power; and if with a low power small objects are seen but not clearly recognized, the higher objective should be applied.

Sometimes one kind of object will be so abundant and predominating as to obscure and hide everything else in the field, as with precipitated phosphates or urates, or leucocytes; it will then be found useful to clear away the superabundant and obscuring material. The phosphates can be easily dissolved and gotten rid of by slightly acidulating the urine before using the centrifuge. The urates can be easily cleared away by heating the urine just before centrifugalizing. Other substances, as leucocytes, it is impracticable to remove.

Sometimes micro-chemical tests are applied, the effects of the addition of reagents being observed under the microscope. A drop or two of the test fluid may be mixed with the urinary sediment before or without applying the cover-glass; or after the cover is applied, a drop of the reagent may be placed at its edge and allowed to flow under.

Various procedures have been suggested for staining the cells and casts, and so making them more conspicuous, as by adding iodine or alizarin solution. These methods have not, however, come into general use. Staining methods may be required for specific purposes, as in examining for bacteria, like the tubercle bacillus or gonococcus; in such cases the centrifuged sediment or a portion of pus present is spread on a cover-glass, dried, fixed, and stained in the usual way for differentiating these or other bacteria.

The objects most commonly seen in microscopical examination of urine are casts of various kinds, cylindroids, mucous threads, epithelium, leucocytes, red blood corpuscles, spermatozoa, bacteria and other vegetable micro-organisms, uric acid, urates, amorphous phosphates, ammonio-magnesium phosphate, calcium oxalate, debris, and foreign particles. These may be recognized by their characteristic appearances as already described. Sometimes consideration of other characteristics of the urine throws light on the nature of doubtful objects; thus, an amorphous granular deposit in alkaline urine is probably phosphatic, in acid urine uratic. Micro-chemical tests may be applied. Thus, leucocytes may be cleared and their nuclei made prominent with acetic acid; urates dissolve with alkalis, or on

adding nitric or acetic acid crystals of uric acid may slowly form; all forms of phosphates dissolve without gas formation on adding acetic or nitric acid.

The following objects are unusually or rarely seen, and may be distinguished by their form, or chemical or micro-chemical tests: xanthin, hippuric acid, leucin, tyrosin, calcium sulphate, indigo granules or crystals, cystin, calcium or magnesium phosphate, calcium carbonate (dissolves with formation of bubbles of gas on adding acid), melanin granules, fat (reacts with Sudan III.), fibrin flakes, hæmatoidin, bilirubin (gives green reaction on adding nitroso-nitric acid), cholesterin, tissue fragments, animal parasites.

Bacteriological examination of the urine is made by the usual cultural methods, the urine being collected with strict aseptic precautions.

VIII. MISCELLANEOUS SECRETIONS AND BODY FLUIDS.

The most important body fluids, from a clinical standpoint, have been considered in the previous chapters. The remaining glandular products or other natural fluids of the body may occasionally be required to be subjected to laboratory investigation for diagnostic purposes.

Saliva consists of a small amount of solids (of which the most notable are ptyalin, mucin, potassium and sodium sulphocyanide, chlorides, phosphates) dissolved in water, along with adventitious epithelium, leucocytes, vegetable micro-organisms, and food particles. It is ordinarily odorless and colorless, and varies in consistency from a thin serous fluid to a more viscid mucinous fluid. It is normally slightly alkaline, but may range from strongly alkaline to acid under morbid conditions. Its specific gravity is ordinarily 1.003 to 1.009. The daily quantity is normally about 1,500 cubic centimetres, but fluctuates greatly under the action of drugs, local or general affections, mental and nervous influences, etc.

Saliva may be obtained for examination by chewing a rubber band and collecting the secretion thus stimulated. The amount that can be secreted in a given time may be thus determined.

To test for ptyalin or the amylolytic power of saliva, add a small amount of saliva to a solution of starch, and after keeping it for a short time at a temperature of about 37° C. test as to the presence of starch, erythrodextrin, and sugar by means of the iodide and copper tests.

To test for sulphocyanides, acidulate saliva, concentrated by evaporation if necessary, with a little hydrochloric acid, and heat with weak ferric-chloride solution; the sulphocyanides cause the formation of a red color.

Certain ingested drugs are freely secreted in the saliva, notably iodides, and their detection in the saliva is a feature of certain clinical tests.

In abnormal conditions, local or general, variations may take place in the various constituents of the saliva; thus, ptyalin may be decreased in fever, urea increased in nephritis. Acid fermentation in the mouth may generate acids and make the saliva acid, with injurious effects on the teeth. Putrefactive processes about the mouth cause a foul odor.

Microscopically, saliva exhibits large, squamous epithelium cells, cast off from the oral mucous membrane; leucocytes and lymphoid cells (salivary corpuscles) that have wandered out of the adjacent tissues and are more or less altered by the action of the saliva; foreign particles derived from food, etc., and large numbers of bacteria.

The vegetable micro-organisms found in the mouth and vicinity are exceedingly abundant, most of them being innocuous, some (as the leptothrix) quite characteristic of this locality, some either actually or potentially pathogenic. The micrococcus lanceolatus, streptococci, and other pathogenic bacteria are frequently present in the mouth of persons in health as well as in those with the corresponding infections. In local infectious conditions the causative bacteria are present, notably diphtheria bacilli, streptococci, pyogenic bacteria, and among pathogenic fungi the oidium albicans, actinomyces, and others. The demonstration of these micro-organisms in pseudo-membranous deposits about the mouth and pharynx, by appropriate cultural and staining methods, is of high diagnostic value.

Bile.—The presence of bile elements in gastric fluid, fæces, and urine has been considered. Bile itself obtained from the gall bladder at autopsy or operation has been scarcely studied pathologically, except as to the presence of bacteria.

Nasal Discharge.—There is very little or none of this normally. In nasal affections it appears, varying in amount and character; similar considerations and methods of examination apply to it as to sputum and catarrhal exudates in general.

Expired Air.—Theoretically the composition of expired air should afford clinical data analogous in all respects to those furnished by the urine; that is, it should yield information as to body metabolism and as to local lesions of the respiratory tract.

Of the three chief end products of katabolism, NH_3 , CO_2 , and H_2O . ammonia, the characteristic representative of nitrogenous katabolism, is chiefly excreted by the kidneys; carbon dioxide, the representative of carbohydrate

katabolism, chiefly by the lungs; while the excreted water is divided between the kidneys, lungs, and perspiration.

The composition of inspired air varies within slight limits, that of expired air within wide limits. Their average composition is about as given in the following table, the figures for expired air being representative of a healthy adult on full diet under conditions of active exercise:

Gas.	BY WEIGHT.			BY VOLUME.		
	Inspired air, per cent.	Expired air, per cent.	Difference.	Inspired air, per cent.	Expired air, per cent.	Difference.
Nitrogen	75.97	76.40	+ .43	78.86	78.80	+ .44
Argon	.97	.97		.80	.80	
Helium	Trace.					
Oxygen	23.01	17.67	- 5.34	20.81	16.00	- 4.81
Carbon dioxide	.05	6.68	+ 6.63	.03	4.40	+ 4.87
Ammonia and other gases.	Traces.	Slightly increased.				
Watery vapor	Variable	Usually to saturation.	+			
	100.00	101.72	+ 1.72	100.00	100.00	
Oxygen	23.01	17.67	- 5.34	20.81	16.00	- 4.81
Oxygen in carbon dioxide	.04	4.86	+ 4.82	.08	4.40	+ 4.87
Total oxygen	23.05	22.53	- .52	20.84	20.40	- .44

$$\text{Respiratory quotient} = \frac{4.87}{4.81} = \frac{6.68 \times .727}{5.84} = \frac{4.82}{5.84} = .90.$$

The principal change in expired air is a decrease of free oxygen and an increase of carbon dioxide and water. The decrease in oxygen (4.81 per cent by volume in the illustration given) represents the amount absorbed into the blood. The increase in carbon dioxide is produced by oxidation processes in the body tissues and excreted by the lungs. It will be observed that more total oxygen (free, and combined in CO_2) is inspired than is expired; that is, more free oxygen is absorbed into the blood (4.81 per cent) than is given out by the blood in the form of carbon dioxide (4.87 per cent); the difference is utilized in the body, amounting in the example given to .44 per cent by volume or .52 per cent by weight.

The proportion of the absorbed oxygen that is given out in the carbon dioxide excreted is called the "respiratory quotient." This is found by dividing the volume of carbon dioxide given out by the blood by the volume of oxygen absorbed by the blood (thus, $4.87 + 4.81 = .9$); or it may be found by dividing the weight of oxygen in the CO_2 (= weight of CO_2 multiplied by .727) by the weight of the absorbed oxygen (thus, $6.68 \times .727 + 5.84 = .9$). The respiratory quotient is increased or diminished as oxidation in the body increases or decreases, and is therefore an expression of body oxidation.

The total amount of oxygen daily absorbed into the blood under ordinary conditions is about 700 to 900 grams in a resting condition, or 900 to 1,100 grams if actively exercising. The daily amount of carbon dioxide given off by the lungs is about 900 grams in the resting condition, 1,000 to 1,800 grams during exercise. The respiratory quotient is about .7 to .8 during rest, .8 to 1 during exercise. These figures all vary according to age, sex, diet, rest or exercise, body condition, and in disease. The amount of water excreted by the lungs is very variable, ranging from 500 to 2,000 grams daily.

These considerations illustrate the class of data that analysis of the expired air would yield with respect to metabolism in disease; but while the subject has been studied for physiological purposes, it has not yet been elaborated in its clinical and pathological relations.

Volatile or gaseous substances in the blood may be excreted by the lungs, as after the use of onions, turpentine, ether, etc.; acetone in acetonæmia, alcohol after its excessive ingestion. Small amounts of ammonia, hydrogen, methane, and nitrogen (in excess of that ingested) are expired. According to some authorities toxic organic substances are normally excreted from the blood into the expired air, which if allowed to accumulate poison and vitiate the atmosphere; other observers have not found proof that such is the case, but attribute the harmful effects of air vitiated by over-breathing and insufficient ventilation to excess of carbon dioxide or watery vapor, or to decrease of oxygen. In renal insufficiency an increase of ammonia is said to be expired.

Necrotic, suppurative, or putrefactive processes in the lungs, air passages, or tributary cavities generate organic and other gases that contaminate the expired air and may impart to it a foul odor; as in pulmonary gangrene, putrid bronchitis, foul rhinitis, decaying teeth, or decomposition of food in an uncleaned mouth. The increase of ammonia and other organic gases in expired air is largely derived from such local conditions.

In ordinary quiet respiration epithelium cells, bacteria, or other particles are not expelled with the breath, though in coughing or sneezing they may be ejected.

Perspiration.—Sweat is one of the three chief excretions of the body, and as such, were it easily investigated, should throw light on body metabolism. The daily amount of sweat is very variable, according to external circumstances and body conditions, but may be stated to average about a litre daily. The perspiration is composed chiefly of water, containing 1 to 2 per cent of dissolved solids,—urea and other nitrogenous bodies (total nitrogen from .02 to .20 grams daily), sodium chloride (the most abundant solid), small amounts of other inorganic and organic salts, fat, fatty acids, etc. The fatty elements originate from the sebaceous glands. The reaction is sometimes alkaline, but ordinarily acid, from the fatty acids present. The odor is due to these volatile acids or fermentative products. Special ex-

creted substances cause the peculiar odor of the sweat from the axillæ, perineo-inguinal region, and between the toes. Fermentative and putrefactive processes from bacterial action may cause sour or foul odors.

The significance of the changes of the perspiration in disease have not been systematically worked out. Urea may be much increased in renal insufficiency, sufficient to give the sweat a urinous odor. Rarely colored sweat is excreted (chromidrosis), red, yellow, blue, black, green, caused by indican products, ingesta, blood and bacterial products, or unknown substances. Phosphorescent sweat, due perhaps to bacterial growth, has been observed.

Semen normally consists of a large proportion of actively motile spermatozoa suspended in a fluid (liquor seminis), together with granular, crystalline, and adventitious matter. The liquor seminis is a muco-albuminous fluid containing sundry chemical ingredients. It exhibits abundant fine granular matter (cellular and protoplasmic debris), and, in small number, cast-off epithelium cells, stray leucocytes, clear "hyaline bodies" from the seminal vesicles, fat and lecithin particles, and small prostatic concretions or "amyloid bodies." After standing, crystals are deposited in semen, especially slender, octahedral crystals of spermin phosphate (Charcot-Leyden crystals).

To determine as to the presence and activity of the spermatozoa, the semen, kept warm, is examined as freshly as possible by placing a drop or two on a warm slide, applying a cover-glass, and examining microscopically with a high power. Thus examined, the spermatozoa may be found absent, or if present they may be motionless, in sterility of the male. Blood may be discharged with the semen, from excessive coitus, or congestive or inflammatory conditions.

For medico-legal purposes suspected stains on clothing, etc., are subjected to examination to determine as to the presence of seminal fluid. If scales can be removed from the stain, they may be macerated and teased in a drop of water or dilute staining fluid on a glass slide, and examined directly with high magnification for the presence of spermatozoa. Or a portion of the cloth containing the stain may be cut out and soaked fifteen to sixty minutes in water or dilute stain (such as Unger's stain, methyl green .2 gm., hydrochloric acid 3 drops, water 100 c.c.)

The fluid in the piece of cloth is squeezed out on a slide and examined with the microscope.

Florence's test for seminal stains, which has been highly commended as a supplement to the above method, is as follows: On a slide mix a drop of a watery extract of the suspected stain with a drop of a solution of iodine 1.65, potassium iodide 2.54, in water 30. The iodide must be in excess. With seminal material this fluid promptly precipitates an abundance of microscopic dark brownish-red, long, slender, rhombic or acicular crystals, single or in rosette or other combined forms. The crystals much resemble hæmin crystals. They soon redissolve and disappear. This reaction is not afforded by other body fluids, so far as known. The ingredient of the semen yielding the crystals has not been definitely determined.

Vaginal Discharges.—The secretion of the normal vagina is a scanty mucous fluid, often acid, containing abundant squamous epithelium cells, with a few leucocytes and bacteria. It is quite strongly germicidal, and not ordinarily favorable to the presence of pathogenic bacteria. The amount is increased during coitus, pregnancy, etc. Spermatozoa are present and demonstrable for a considerable period after coitus.

Leucorrhœal discharges are of the nature of catarrhal fluids, and are of a muco-purulent or purulent character, containing leucocytes in proportion to the suppurative process going on. In local inflammations and infections the causative bacteria are demonstrable by staining or cultural methods, as gonococci, staphylococci, streptococci, etc. A protozoan, the *trichomonas vaginalis*, has been frequently found in the vaginal fluids, especially in some localities; it appears to have little or no pathogenic action in the vagina, and has been also found in the fæces and urine. Large shreds or membranes from the vaginal mucosa are sometimes discharged.

Uterine Discharges.—*Menstrual fluid* consists chiefly of blood, with some vaginal secretion, vaginal and uterine epithelium cells, and granular and fatty débris from the uterine mucosa.

The *lochia* for the first two or three days after labor are abundant, thin, and red, and chiefly composed of blood, with vaginal epithelium and decidual cells. The amount steadily decreases, the red blood cells diminish, the leucocytes and epithelium increase, and the color changes to rusty-brown, gray, or white.

Bacteria are always abundant. The odor of uncontaminated lochia is bloody; in the presence of decomposing clots or retained portions of the afterbirth or septic processes the odor becomes fetid; sometimes an ammoniacal odor develops from ammoniacal fermentation, which is innocuous.

In septic or infected conditions of the uterus the discharges become purulent, and the causative bacteria may be demonstrated. Masses, shreds, or fragments of tissue may be expelled from the uterus, consisting of clots, moles, embryos, decidua, dysmenorrhœal membranes, etc.

Amniotic fluid rarely exhibits abnormalities, which although interesting are scarcely of practical importance.

Milk consists of a large number of minute fat globules suspended in a watery solution of proteids, lactose, and salts. Cellular elements are ordinarily absent; bacteria are usually present. The composition of normal human and cow's milk varies somewhat, but averages about as follows:

	Human milk.	Cow's milk.
Proteids (chiefly casein).....	2.0	3.8
Fat.....	3.5	4.0
Lactose.....	5.8	5.0
Salts (phosphates, etc.).....	.2	.7
Total solids.....	11.5	18.0
Water.....	88.5	87.0
	100.0	100.0

Human milk is alkaline in reaction, has a specific gravity of 1.026 to 1.035 (averaging about 1.032), and in twenty-four hours amounts to $\frac{1}{2}$ to $1\frac{1}{2}$ litres. Numerous substances ingested by the mother or elaborated by maternal metabolism are excreted in the milk.

Abnormal changes in the milk may be caused either by local morbid conditions of the mammary apparatus or by general systemic disturbances. In inflammatory, suppurative, or hemorrhagic conditions of the breast, leucocytes and red blood corpuscles may appear in the milk. Bacteria in the milk may originate from the maternal tissues or be introduced after withdrawal. Some of these bacteria are pathogenic, as staphylococci, streptococci, typhoid bacilli, micrococcus lanceolatus, etc., originating

in the corresponding infections; others induce fermentative changes in the milk, especially formation of lactic acid from lactose and coagulation of casein; many micro-organisms in milk are indifferent and harmless. The agglutinins of typhoid fever may appear in the milk.

Variations in the composition of milk are caused by various general conditions of the mother, as fevers, lowered general nutrition. The changes in special diseases have not been much studied, and analyses of milk are made chiefly in connection with infant feeding. Milk for examination is collected with a breast pump. A drop of the milk under the microscope exhibits a large number of minute rounded fat globules 1 to 5 micromillimetres in size. To search for cellular elements, leucocytes, etc., centrifuge the milk and examine the sediment under the microscope. Bacteria may be searched for by staining the centrifuged sediment, cultural methods, or animal inoculation.

The two chief points to be determined in the analysis of milk are the specific gravity and the amount of fat. These two data ordinarily afford a sufficiently good idea of the nutritive strength of the milk, and from them the total solids can be approximately calculated.

The *specific gravity* of milk is determined by the pyknometric method, or by a sensitive "lactometer" on the hydrometer principle, especially constructed for the purpose. Temperature corrections should be made.

The amount of *total solids* is determined by drying a definite quantity of the milk and weighing the residue after its weight has become constant. The total solids can also be calculated (for cow's milk) by adding 1.2 times the percentage of fat to one-fourth of the specific gravity at 15° C.; the sum is approximately the percentage by weight of the total solids.

The estimation of the amount of *fat* in milk is one of the chief procedures of milk analysis, since the other solid constituents usually correspond closely with the fat in amount. For this purpose a number of simple methods of sufficient approximate correctness have been devised, of which Babcock's sulphuric-acid centrifugal method is in common use. In Babcock's test equal carefully measured amounts of milk and strong sulphuric acid are thoroughly mixed; the acid dissolves all but the fat; on centrifugation in a special bottle with a graduated neck, the fat

is forced to the top and from the graduation its weight percentage is directly read off. The relation between the volume and the weight of the butter fat is such that at 50° C. each 1 per cent by weight indicated on the graduated scale occupies 1.1428 per cent by volume of the amount of milk originally taken for the test. With care this method yields quite accurate results.

By using a mixture of equal parts of pure amyl alcohol and strong hydrochloric acid (of specific gravity of 1.16 or over), in amount equal to one-fifth of that of the milk used, in addition to the sulphuric acid, a sharper separation of the fat is obtained (Leffmann-Beam method).

Commercial establishments use a large centrifugal machine for the Babcock test, but for clinical purposes it is more convenient to use a smaller centrifugal tube adapted to the ordinary laboratory centrifuge (Fig. 34). To make the test, the requisite amount of milk, 5 cubic centimetres, is accurately measured in a pipette and introduced into the tube. One cubic centimetre of the amyl-alcohol and hydrochloric-acid mixture is then added and thoroughly mixed; this procedure may be dispensed with, but it facilitates reading the final result. Strong sulphuric acid (specific gravity 1.83 to 1.84) is added till the tube is filled to the shoulder, and the fluids are thoroughly mixed. When the mixture becomes dark and homogeneous, add sulphuric acid to the top of the graduated scale, and again mix the fluids. Revolve the tube in the centrifuge for a couple of minutes. The liberated fat is concentrated at

the top of the tube, and its amount is read off at once from the scale. If from cooling the fluid contracts below the graduation, the tube should be placed in hot water until the fluid expands sufficiently to enable the result to be read. The column of fat should be free from bubbles and undissolved casein.

Commercial sulphuric acid of specific gravity 1.82 to 1.83 may be used if the tube containing the milk-acid mixture is kept in hot water at about 95° C. for a few minutes before centrifugation.

Proteids in milk are determined gravimetrically after precipi-



FIG. 34.—Centrifugal Tube and Pipette for Estimation of Fat in Milk. (Bausch & Lomb.)

tating them, or estimated by multiplying the amount of nitrogen found by Kjeldahl's method by 6.33.

Lactose in milk is determined quantitatively by the polarimetric or copper-reduction methods.

Articular Fluids.—Synovial fluid is normally a muco-serous fluid, glairy from the presence of mucin. In articular lesions, inflammatory, suppurative, etc., it undergoes corresponding alterations variously exhibiting fibrinous coagula, pus and leucocytes, blood and erythrocytes, and bacteria. These foreign elements are demonstrable with the aid of the centrifuge, the microscope, and cultural methods.

Cerebro-spinal Fluid.—Examination of this fluid is capable of affording valuable diagnostic information in cases of suspected cerebro-spinal meningitis and other affections.

The fluid is obtained for examination by means of "lumbar puncture," as follows: With the patient sitting, lying on the side, or doubled face downward over the knees, the body and back being bent forward as far as possible, with strict asepsis, and under local or if necessary under general anæsthesia, a strong aspirating needle is introduced in the interval between the second and third, third and fourth, or fourth and fifth lumbar vertebræ. A good guide for the insertion of the needle is the line connecting the crests of the ilia. The needle, introduced in this line and about a centimetre to one side of the spinous processes, is pushed forward and slightly inward and upward until its point enters the subarachnoid space, which is shown by escape of the fluid. The depth to which the needle must be passed varies from two or three centimetres in infants to eight or ten in adults. The syringe may be attached to the needle as a handle. The fluid may be allowed to flow out spontaneously to the extent of a few cubic centimetres, or may be withdrawn by very gentle suction with the syringe, and is collected in a sterile receptacle. The needle and syringe should be sterile, but should not contain any antiseptic at the time of use.

Normal cerebro-spinal fluid is a clear, limpid, non-coagulating, colorless or slightly yellowish (sometimes accidentally tinged with blood), alkaline serous fluid containing about 1.3 per cent of various solids in solution, and of a specific gravity of 1.005 to 1.007. The most abundant solids are albumin (normally about .1 per cent or less) and chlorides. A few endothelium cells,

leucocytes, and fibrin filaments may appear microscopically. As to the amount of fluid normally obtainable by lumbar puncture no definite statement can be made, but ordinarily several cubic centimetres can be easily and safely withdrawn.

In abnormal conditions the cerebro-spinal fluid may become modified in consequence of inflammatory, suppurative, hemorrhagic, and dropsical processes, etc., in the cerebro-spinal structures. The chief abnormalities of practical diagnostic significance consist in variations of amount, appearance, specific gravity, quantity of albumin, the presence of pus and leucocytes, of bacteria, and of blood and erythrocytes.

The amount of cerebro-spinal fluid obtainable is increased in hydrocephalus, meningitis, etc., in which there is an increased exudation of fluid and increased tension. It is decreased or unobtainable altogether when fibrinous or purulent exudations, adhesions, or pressure of tumors obstruct the passages along which the fluid flows.

In fibrinous or suppurative meningitis the cerebro-spinal fluid becomes turbid, in hemorrhagic conditions it is bloody, in serous meningitis, tubercular meningitis, hydrocephalus, etc., the fluid is clear and colorless. In local inflammatory (but not in other) conditions the fluid clots, either in the form of a delicate fibrin network (tubercular meningitis) or as solid coagula. Simple inspection of the cerebro-spinal fluid therefore affords valuable diagnostic information.

As a rule the amount of albumin, and with it the specific gravity of the fluid, is greater in meningitis than in non-meningitic conditions (as hydrocephalus), the inflammatory processes or exudations introducing a larger proportion of proteids than is present in the normal fluid or simple transudations.

In suppurative meningitis pus appears in the fluid, varying from a slight admixture to almost pure pus. In such cases the fluid is cloudy, and microscopically exhibits leucocytes in numbers corresponding to the proportion of pus present.

In infectious cerebro-spinal conditions the causative bacteria are present, and their demonstration, which is possible in most cases, establishes the specific diagnosis. The bacteria chiefly responsible for meningitis are the tubercle bacillus, diplococcus meningitidis intracellularis, and micrococcus lanceolatus, with staphylococci, streptococci, typhoid bacilli, and others in a small

proportion of cases. Of these the tubercle bacillus is associated with non-suppurative meningitis, the cerebro-spinal fluid being clear and serous, while the other bacteria cause suppurative inflammations and a cloudy, purulent fluid.

Blood, manifested by the color, microscopical presence of red blood corpuscles, and chemical reactions, appears in the cerebro-spinal fluid in hemorrhagic conditions, aside from traces that may be introduced accidentally in the process of lumbar puncture. In cerebral and intraventricular hemorrhage, a large admixture of blood with the cerebro-spinal fluid takes place; while in extradural bleeding little or no blood enters the fluid.

The examination of the fluid obtained by lumbar puncture is accomplished by inspection, microscopical methods, bacteriological methods, or chemical tests.

Simple *inspection* reveals much information as to the color, clearness, or turbidity of the fluid, and as to fibrin formation, with all that these imply.

Microscopical examination is for the purpose of investigating as to the bacteria and cells present. Unless the fluid is very thick and turbid, the sediment should be concentrated and collected with the centrifuge, and then be examined either fresh or after staining. For the demonstration of bacteria the various appropriate staining methods should be employed.

Tubercle bacilli, if present, are often difficult to demonstrate. If they cannot be found after the use of the centrifuge, the fluid should be allowed to stand in the cold for several hours until a fibrinous network or coagulum forms. These fibrin filaments, in which the bacilli are apt to be entangled, are removed with a platinum loop, spread on a cover-glass, fixed, and stained in the usual manner for tubercle bacilli.

Bacteriological examination of the cerebro-spinal fluid, in cases in which the microscopical examination is not regarded as sufficient, is carried out by cultural methods or animal inoculation in the usual manner. For this purpose, the fluid must have been collected and preserved with absolute asepsis, and without contamination with antiseptics.

Chemical tests are rarely called for, but if desired can be carried out, as for the amount of albumin, presence of blood, specific gravity, etc.

IX. PATHOLOGICAL FLUIDS.

This class embraces fluids produced only in consequence of pathological processes. These fluids may be classified according to their composition, as serous, sero-mucous, mucous, mucopurulent, purulent, etc., or according to their cause, as inflammatory, catarrhal, suppurative, cystic, etc. The pathological body-fluids will here be classified and considered as follows:

Transudates, dropsical. Cyst fluids. Exudates, inflammatory: serous, fibrinous, sero-fibrinous, cancerous. Hemorrhagic, chylous, and fatty effusions. Pus. Catarrhal exudates. Granulation exudates. Necrotic fluid.

Transudates.—This term is applied to dropsical effusions into the serous cavities or areolar tissues of the body, in contradistinction to exudates of similar appearance and location produced by inflammatory processes. The possibility of making a distinction between transudates, cyst fluids, and exudates may aid in making a diagnosis between dropsical, cystic, and inflammatory conditions. Transudates most often occur in the subcutaneous tissues in general anasarca, in the peritoneum in ascites, in the pleura or pericardium in hydrothorax and hydropericardium, or the cerebro-spinal cavities in hydrocephalus. Transudate fluid is readily collected by means of the trocar and canula, or from the subcutaneous tissues by incising the skin and collecting the fluid as it drains away.

Dropsical transudates usually consist of a sterile, clear, light-yellow, serous fluid, of alkaline or neutral reaction. Chemically they are composed of a solution of about 1 to 5 per cent of solids in water. The solids consist of various mineral and organic substances, among which sodium chloride and proteids are much the most abundant. Variations in the composition of transudates consist chiefly in variations in the proportion of proteids; and the chief difference between transudates and serous exudates is in the larger amount of albumin in the latter. The proportion of albumin in transudates ranges usually from 2 or 3 per cent down to small amounts, while in exudates it is usually from 3 to

8 per cent. The specific gravity of transudates is usually 1.005 to 1.015, that of exudates 1.018 to 1.030; as the variations in specific gravity depend chiefly on variations in the amount of albumin, the specific gravity may be taken in general as an index of the proportion of albumin and as a distinguishing characteristic between transudates and exudates.

Microscopically transudates may exhibit a few leucocytes and endothelium cells, sometimes showing fatty degeneration. Cholesterol crystals sometimes appear. On standing a fibrinous nubecula similar in appearance to that of urine sometimes forms. Amœbæ have been seen in ascites fluid.

At times transudates may be tinged reddish or brown with blood or transformed blood pigment, or green with bile pigment.

Cyst fluids vary widely in character according to the conditions which produce them, being serous, mucoid, colloid, fatty, etc. The fluid is obtainable by aspiration.

Retention cysts originate from an accumulation of secreted fluid in a glandular cavity whose outlet has become occluded. The fluid in such cysts partakes to a certain degree of the characters of the corresponding normal secretion of the gland, and the identification of the origin and character of the cyst depends in general on the detection in the fluid of the specific gland cells or secreta. Thus, the presence of trypsin, shown by its power of digesting coagulated albumin in alkaline media, would indicate a pancreatic cyst; urinary ingredients and renal cells would appear in hydronephrosis; mucinous material in cysts of mucous glands, colloid material in goitrous cysts, milk or its products in galactoceles, fatty matter in sebaceous cysts; hair, teeth, sebaceous matter, etc., in dermoid cysts. In the course of time, however, the gland substance may be entirely destroyed and the retained fluid may lose the specific glandular ingredients.

Ovarian Cyst Fluid.—The recognition of this fluid may be of importance in diagnosing between ascites and ovarian cysts. The fluid from these cysts is usually clear, colorless, yellowish, or brownish; the presence of blood elements gives a red or brown color. The fluid varies from a thin, limpid consistency to a thick, viscid, and gelatinous consistency, and ranges in specific gravity from 1.007 to 1.026; the variations in consistency and specific gravity depend chiefly on the presence of colloid, mucoid, and proteid material in varying amount. Metalbumin or paralbu-

min is a characteristic proteid ingredient of most ovarian cyst fluids, as distinguished from other cysts. Microscopically, ovarian cyst fluid may exhibit leucocytes, epithelium cells (rounded, elongated, ciliated, or squamous), Drysdale's corpuscles, cholesterin crystals, red blood corpuscles, rounded colloid bodies. The epithelium cells may be fatty or granular, and their character indicates the nature of the cyst walls. Drysdale's corpuscles are rounded, non-nucleated, granular cells about the size of leucocytes; the protoplasm between the granules is clear and transparent; the granules are distinctly outlined, insoluble in acetic acid, and insoluble or incompletely soluble in ether; Drysdale's corpuscles are regarded as very characteristic of ovarian cysts, and are perhaps freed nuclei.

In dermoid ovarian cysts dermoid elements are present.

The chief distinguishing characters of ovarian cyst fluid as compared with ascites fluid are the colloid consistency, the presence of columnar or ciliated cells and of Drysdale's corpuscles. The distinction cannot always, however, be made.

Exudation cysts arise from excessive effusion into normal cavities, as in hydrocele.

Hydrocele fluid in its characters frequently approaches those of inflammatory exudates, containing a large proportion of proteids and often with a high specific gravity, 1.015 and upward. The fluid is usually serous, limpid, clear, amber or yellow in color; frequently great numbers of large macroscopic glistening plates of cholesterin are present, and sometimes the fluid is dark-colored and cloudy from the presence of transformed blood elements. A very few cases of chylous hydrocele or chylocele have been observed, in which the sac contained a milky chylous fluid.

Disintegration cysts contain fluid varying in character, resulting from necrotic liquefaction of solid tissues.

Parasitic cysts are produced by parasites, such as echinococci, cysticerci, trichinæ, and others. Most varieties contain little besides the parasitic forms, but echinococcus or hydatid cysts also contain a large amount of fluid.

Hydatid fluid is clear and serous, with a specific gravity of 1.006 to 1.010, and contains very little or no albumin; microscopically, the detection of shreds of cyst-membrane, scolices, or hooklets is diagnostic.

Inflammatory exudates, produced by inflammatory processes,

may be serous, fibrinous, sero-fibrinous; when associated with hemorrhagic or suppurative processes the product becomes bloody or purulent, and when involving a mucous surface mucinous elements are introduced.

Serous exudates, best exemplified by the effusion in serous pleuritis and blisters, are usually limpid, clear, colorless or yellowish, containing 3 to 8 per cent of proteids, and have a specific gravity of 1.018 to 1.030; they sometimes coagulate on standing. They resemble dropsical transudates, except in their higher specific gravity, their greater proteid content, and their tendency to coagulation. Microscopically the centrifuged sediment exhibits a few leucocytes and endothelial cells, usually fatty. Bacteria may be demonstrable by staining, cultures, or animal inoculation.

Fibrinous exudates, consisting of white, somewhat adhesive, fibrinous coagula, are produced in acute plastic inflammations, especially of the peritoneum and pleura. Fibrin has rather characteristic staining properties, by which it may be distinguished; it is oxyphile, and stains with acid stains, while mucin is basophile. After fixing with alcoholic mercuric-chloride solution (5 per cent) fibrin stains red with the triple stain (mucin green). Fibrin retains a blue stain in the Weigert-Gram method, which is as follows: Fix and harden in alcohol. Stain five to fifteen minutes in a strong solution of gentian violet in anilin-water. Wash in .6-per-cent sodium-chloride solution. Dry with filter-paper on a slide or cover-glass. Transfer to Gram's iodine solution two or three minutes. Dry with filter-paper. Decolorize sufficiently in anilin oil 2 parts, xylol 1 part. Wash in xylol and mount.

Sero-fibrinous exudates, as from peritonitis, pleuritis, arthritis, consist of a serous exudate in which are suspended fibrinous coagula and particles.

Cancerous exudates, such as may be produced in malignant disease of the pleura or peritoneum, have as a basis a serous exudate usually containing blood and cells or fragments of tissue from the neoplasm. The fluid usually has a large proteid content (3 to 6 per cent) and a high specific gravity (over 1.020). Blood, fresh or transformed, is often present. Abundant cells or fragments of tissue, sarcomatous or carcinomatous, may be present in the fluid and aid in diagnosis; but the identification

of isolated cells as sarcoma or carcinoma cells is always a difficult and uncertain matter. Sometimes the free cells undergo fatty degeneration and give the fluid a chyloform or fatty character.

Hemorrhagic Effusions.—Blood may become mingled with and add its characters to any form of transudate or exudate, in consequence of traumatic or ulcerative conditions, etc. If fresh, the color will be bright-red; if stale, the decomposed and transformed blood pigment produces a brown or dark color. The presence of fibrin factors causes some tendency to clotting. The tests for blood will be positive, and microscopically red blood corpuscles may appear. Bloody effusions in the pleura, peritoneum, or tunica vaginalis are especially due to malignant disease, traumatism, or tuberculosis of the part.

Chylous Effusions.—In numerous instances the fluid withdrawn from cases of ascites, and in a very few instances effusions into the pleura (chylothorax), pericardium, and tunica vaginalis (chylocele), have been found to be white, opaque, and milky—true chylous fluid. Microscopically, fat particles are seen suspended in a state of very fine granular division, with a few red blood corpuscles, leucocytes, and endothelium cells. On standing, a creamy layer rises to the top, and a gelatinous coagulum settles to the bottom. The reaction is alkaline or neutral, specific gravity 1.007 to 1.023, total solids 5 to 12 per cent, proteids up to 6 per cent. The effusion is caused by a leakage of chyle into the serous cavity involved, either from rupture of lymphatic vessels, or obstruction of the thoracic duct, or filariasis. Blood may also be present.

Fatty effusions (also called chyloform, chyloid, adipose, oily, and lacteal effusions) resemble true chylous effusions, but the fat is not so finely divided, occurring in larger droplets or coalescing in masses of fluid oil. These effusions are produced by fatty degeneration of the cells in the fluid, usually endothelium cells or carcinoma cells, less often leucocytes or fibrin.

Pus is the specific product of suppuration. Pure typical pus is produced by uncomplicated suppuration in the solid tissues, as in acute abscesses, and exhibits characteristic features. When the suppurative process is associated with serous or catarrhal inflammation, hemorrhage, or necrotic processes, the resulting product is of a mixed type, containing pus mixed with

serous exudate, mucus, blood, and disintegrated tissue, forming sero-purulent, muco-purulent, sanguino-purulent exudates, etc. Pure pus may, however, be generated from serous or mucous surfaces.

Typical and pure pus, such as is found in acute abscesses, consists essentially of a crowded mass of leucocytes suspended in a fluid (pus serum), together with the bacteria or other parasites causing the suppuration, and with more or less granular, amorphous, and degenerative materials. The color ranges from grayish to light-yellow or greenish. The bacillus pyocyaneus produces bright-green pus, the admixture of blood gives a brown color. The consistency is creamy, varying from a quite fluid condition to a thick, viscid, semi-solid mass. The odor is disagreeable and may be very foul and repulsive. The reaction is alkaline, the specific gravity 1.025 to 1.040. After standing or centrifugalizing, the corpuscles settle, leaving the pus serum supernatant.

The pus serum is a solution of about 8 to 10 per cent of solids, which are chiefly proteids, with a small amount of fatty material. The leucocytes of pus are almost exclusively of the polynuclear neutrophile variety; an occasional large mononuclear leucocyte is present, while in gonorrhœal pus and asthmatic sputum eosinophile leucocytes are often comparatively abundant. Amorphous granular or flaky material, fat particles, etc., are seen in greater or less amount, especially in abscesses of long standing. Crystals of cholesterin, fatty acids, or triple phosphate may appear in pus from chronic abscesses, and red blood corpuscles or hæmatoidin may appear when there has been an admixture of blood. Fragments of necrotic tissue are sometimes present.

In pus from circumscribed abscesses ordinarily only the bacteria causing the suppuration are present, while in pus from localities exposed to contamination adventitious bacteria may be abundant. The pyogenic bacteria that occur in pus are numerous, the most common forms being the staphylococci and streptococci, less common being the bacillus pyocyaneus, bacillus coli communis, gonococcus, micrococcus lanceolatus, tubercle bacillus, and numerous others. Actinomycetes, streptothrix, blastomycetes, the amœba coli, psorosperms, filariæ, trematodes and their ova, and other fungous and animal parasites are at times

found in pus. Theoretically but scarcely in practice pus may be generated by irritants independently of bacterial action.

Pus from chronic or old abscesses varies considerably in some features from the typical pus of acute suppurations. The leucocytes are frequently fatty or otherwise degenerated, diminished in number, and at times almost absent; they often do not take stains in the normal way. There may also be an abundance of amorphous cheesy material, which seems to take the place of leucocytes. The bacteria are sometimes diminished in number, and the pus may even become sterile. Crystalline, granular, and fatty material may be abundant.

In exudates of mixed type, the pus is mixed in various proportions with serous, mucinous, and other elements. The amount of pus present is proportionate to the quantity of leucocytes, which may be estimated from the amount of sediment thrown down by the centrifuge or from the appearance of microscopical specimens.

Examination of Pus.—The color, odor, consistency, and general appearance are obvious to simple inspection. Sedimentation or centrifugation affords an idea of the quantity of leucocytes present. Examination of fresh unstained pus under the microscope reveals leucocytes, crystals, amorphous material, amœbæ, filariæ, fungi, or other parasites. The leucocytes can be studied by the same methods of fixing and staining (triple stain, etc.) as are employed in the case of the blood. The chief clinical importance of pus ordinarily depends on the kinds of bacteria present. The determination of the bacteria in pus is accomplished by staining methods or by the usual cultural methods or animal inoculation.

The *methods of staining pus* are similar to those of staining sputum. By means of the platinum loop a drop or two of the fluid to be examined is spread out in a thin layer on a slide or cover-glass and allowed to dry; or a drop of the pus is squeezed out into a thin, even layer between two cover-glasses, which are then slid apart and dried, leaving a thin film on each cover. In preparation for staining, the cover-glass spreads are "fixed" by flaming or passing them three times through a Bunsen or alcohol flame at moderate speed and brief intervals. The films are then stained for a few seconds or minutes by covering them with or floating them on the staining fluid employed. For staining most

bacteria Loeffler's methylene blue, dilute carbol-fuchsin, gentian violet, and Gram's method are the best. For the tubercle bacillus Gabbet's method (page 130) is employed. For the gonococcus Loeffler's methylene blue or Gram's method is used. Special methods may be employed for special purposes.

Catarrhal Exudates.—The products of inflammatory processes affecting mucous membranes correspond to exudates in general, but are modified by the introduction of special elements, notably mucin, epithelial cells, and many adventitious bacteria. Sputum, a typical catarrhal discharge, has been considered in detail; the discharges from other mucous surfaces are in general similar to sputum. The exudate varies according to the form of inflammation. In the initial stage of catarrhs (as in acute rhinitis) the exudate may be purely serous, thin, clear, and limpid; ordinarily, catarrhal exudates contain much mucin, affording purely mucous discharges or mixed muco-serous or muco-purulent fluids; in the presence of suppurative processes a proportionate amount of pus is introduced into the exudate, or even pure pus is produced. Foul putrefactive products and gases are generated in necrotic, putrid, or stagnant cases. A fibrinous exudate in the form of a pseudo-membrane is produced on mucous membranes by the diphtheria bacillus, streptococci, staphylococci, micrococcus lanceolatus, or in debilitated conditions. In mycotic catarrhs, as in thrush, a fungous growth develops on the mucous surface.

Catarrhal exudates therefore consist of serous, mucoid, purulent, bloody, putrid, or fibrinous elements in pure form or mixed in variable proportions.

The color varies from colorless, grayish, yellowish, greenish-yellow, brown, rarely bright-green or other color. The consistency, homogeneity, and odor vary according to circumstances, much as in the case of sputum.

Leucocytes or red corpuscles are present in proportion to the extent of suppuration or bleeding. Epithelium cells from the mucous membrane involved are present, either the surface cells of the part or the deeper germinal cells immaturely and prematurely cast off; they are usually more or less swollen, altered, or degenerated. Bacteria of many varieties and in enormous numbers are often present, though in some cases they may be limited to the variety causing the trouble.

The purpose of the examination of catarrhal exudates is partly to determine as to the presence of leucocytes, blood corpuscles, or epithelium, but ordinarily especially to determine the bacteria causing the inflammation in the case under investigation. For this purpose the usual methods of staining and microscopical examination, cultural methods, etc., are employed as in the case of sputum or pus. Special methods are employed for special micro-organisms, as for tubercle bacilli, gonococci, and diphtheria bacilli.

Granulation Exudates.—The initial effusion from wounds consists of blood and lymph from severed vessels. If infection, inflammation, or suppuration subsequently develops, the corresponding inflammatory or purulent exudates are generated in abundance. Sterile and uninfected granulating wound and ulcer surfaces yield a scanty amount of whitish fluid, of a creamy or sticky consistency, odorless, or nearly so, and entirely free from bacteria; microscopically numerous leucocytes with amorphous matter are seen.

Necrotic Fluid.—The fluid materials produced by the liquefactive disintegration of necrotic tissue vary in their ultimate fate and character. If isolated by solid tissue walls and not infected, cyst-formations may result, with fluid or gelatinous contents. If, as is frequently the case, infection occurs, foul putrid suppuration results, with exceedingly repulsive discharges, as in pulmonary gangrene.

Frequently in operation wounds through a thick panniculus adiposus (especially for hernia) that are sterile and infected throughout, necrosis of the fatty subcutaneous tissues occurs, with the production of a fluid that much resembles pus and therefore gives rise to needless alarm. This form of necrotic fluid is creamy, dark-colored, and contains leucocytes, amorphous material, and sometimes masses of necrotic tissue; bacteria are absent, and the condition is not in any way the result of infection.

X. CALCULI.

Scarcely any part of the body, either solid tissues or hollow viscera or ducts, is exempt from the formation of hard calculous bodies. The calculi of the gall bladder and urinary organs are much the commonest and most important clinically. In special situations calculi may be composed of special substances, as cholesterin and bile pigments in the gall bladder, uric acid, calcium oxalate, etc., in the urinary organs. The generality of calculi are composed of insoluble earthy salts mingled with organic matter; of the earthy material calcium phosphate ordinarily greatly preponderates, with calcium carbonate, ammonio-magnesian phosphate, magnesium phosphate, magnesium carbonate, and calcium sulphate in amounts varying from zero or traces up to considerable proportions; cholesterin or fatty matter is sometimes present. Most but not all calculi are formed by the deposition of solid material on a nucleus or matrix of organic matter or of some foreign body, as fibrin or mucus, inspissated secretions, bacterial or fungous growths, caseous or necrotic tissue, or a fragment of another calculus. Frequently calculi consist of concentric lamellæ which sometimes differ from one another in composition. The size and form vary greatly; in number they may be single or multiple; when a number are in contact, the apposed surfaces are smooth and faceted.

Calculi can be considered both with reference to their situation and their composition.

In varicose veins and vascular tumors concretions (*phleboliths*) occasionally occur, composed of the usual calcareous and earthy deposits mingled with fibrin. Calculous deposits, chiefly calcium phosphate, occur rarely in the thoracic duct, receptaculum chyli, and lymphatic glands, in the latter especially when caseous.

Salivary concretions (*sialoliths*) of the usual calcareous composition occur occasionally in the submaxillary gland or its duct, less often in the sublingual or parotid, rarely in the small mucous

glands of the palate, and are similar in composition to the tartar of the teeth.

In the *tonsils* calculi sometimes form from inspissation and calcification of the contents of obstructed crypts.

Calculi are rare in the *stomach*, being formed chiefly by an aggregation of insoluble ingested substances, as lime and magnesia salts, salol, fatty material, resinous substances, etc. *Aegagropili* consist of hair, ingested by the mouth and matted together in balls; they occur in the *œsophagus*, *stomach*, or *intestine*, and are commonest in herbivora, but are rarely found in man.

Enteroliths, or intestinal calculi, are formed either (a) from insoluble ingesta like salol, lime or magnesia salts, or fat, (b) by the deposition of calcareous matter on some food particle (like seeds) or a bit of hard *faecal* matter as a nucleus, or (c) most commonly by the inspissation of *faeces* and infiltration with earthy salts (*coproliths*). They are formed chiefly in the *appendix*, *cæcum*, or *saccular* pockets of the *colon* or *rectum*. They often contain large amounts of triple phosphates.

Calculi of the usual calcareous composition sometimes form in the ducts of the *pancreas* and may attain large size.

Biliary calculi are frequently observed. They vary in number from one to hundreds or even thousands, and in size from that of the distended gall bladder to fine granules. They are composed of the ingredients of the bile, mixed in varying proportions, sometimes one, sometimes another substance preponderating. The chief materials of which gall stones are formed are *cholesterin*, *bile pigments*, *earthy phosphates* and *carbonates*, and occasionally *biliary* or *fatty acids*. Traces of *metallic substances* are sometimes present. Most gall stones contain 70 to 90 per cent of *cholesterin*, and some are almost pure *cholesterin*. Some of them have a nucleus of *bacteria* or other particles, and some are laminated and heterogeneous in composition. The *cholesterin* and *pigment calculi* are rather soft, the *calcareous stones* harder and firmer. The *bile pigment* present, according to its amount and kind, gives gall stones a brown, or exceptionally a black, green, blue, or yellowish-red color. *Cholesterin* and *calcareous concretions* nearly free from *bile pigment* may be white or gray.

Occasionally *fibrinous concretions* occur in the *peritoneal cav-*

ity, either loose or attached by a pedicle. They have been observed larger than a billiard ball. They originate from the fatty omental appendages as nuclei, which become necrotic or calcified and then covered with proteid or fibrinous laminæ. Lithopædia also form calcified masses.

Calculi of the nasal fossæ and accessory sinuses (*rhinoliths*) are usually single, weighing from 2 to 10 grams. They consist mostly of calcium phosphate, with often notable proportions of calcium carbonate and magnesium phosphate. Most rhinoliths are formed on a nucleus of some foreign body, as seeds.

Calculi rarely form in the bronchi (*bronchololiths*) or pulmonary cavities, from deposition of calcareous material about a nucleus of some kind, as dried sputum, bits of tissue, etc.; fatty bronchololiths have also been known. *Pneumoliths* are formed by the deposition of calcareous matter in the substance of the lung, most frequently in caseous tuberculous foci, but sometimes in healthy tissue. Calcareous deposits are sometimes laid down in the pleural membrane or free calculi may be formed in the pleural cavity.

Calculi in the *skin* occur rarely, resulting from inspissation and calcification of the contents of sebaceous cysts (*seboliths*). They are composed of fatty and calcareous material. They may also occur in the subcutaneous tissues.

Urinary calculi may form in any portion of the urinary tract, renal parenchyma, renal sinus, ureter, bladder, or urethra. They vary widely in size, from small granules (gravel) up; in one case a calculus weighed 1,596 grams. They are usually single, but they may be multiple, up to several hundred in number. They are usually formed on a nucleus of inspissated mucus or pus, bacteria, epithelium cells, crystals, foreign bodies, etc. Sometimes they are laminated, and sometimes a calculus is composed of different substances in different parts. In composition urinary calculi present considerable variation. The commonest varieties are those composed of uric acid, urates, calcium oxalate, or phosphates, respectively; rarely urinary calculi are composed of xanthin, indigo, cystin, calcium carbonate, fat or soap (*urostealiths*), cholesterin, or fibrin.

Uric-acid calculi are the commonest form of primary urinary calculi, and frequently serve as nuclei on which large concretions are formed by deposition of phosphates or oxalate. They occur

especially in children, old age, and the gouty, and may attain large size. They are usually formed in the kidney.

Urate concretions may be developed in the kidneys of children. Urates do not often form the only ingredient of calculi, but are usually mixed with uric acid or calcium oxalate.

Calcium-oxalate calculi are sometimes nearly pure, sometimes mixed with uric acid or urates, and are formed in acid urine similarly to uric-acid calculi.

The phosphatic calculi are composed of calcium phosphate or ammonio-magnesian phosphate, usually mixed, but sometimes either one alone. Phosphatic concretions are formed especially in cystitis with ammoniacal urine, and hence chiefly originate in the bladder. They are usually formed on other calculi (uric acid or oxalate) as a nucleus, the latter setting up a cystitis that favors the precipitation of phosphates. They may attain large size.

Cystin and calcium-carbonate urinary calculi are rare. Xanthin, indigo, cholesterol, and fatty or soapy concretions in the kidney or urinary passages have been noted only in a very few instances (one or two to a dozen or so each). Hardened masses of inspissated fibrin or blood clots have also been observed.

In four or five cases calculi resembling urinary concretions have been found at the *umbilicus*, apparently formed in a patent urachus communicating with the bladder. Sebaceous concretions have also been found at the navel.

Urethral calculi are usually formed in obstructed or traumatic conditions of the urethra. They may occur in any part of the canal from the meatus to the prostate, and usually consist of calcareous and phosphatic deposits on fragments of calculi from the bladder, or other foreign bodies. Calcium carbonate and uric acid may also occur in the deposits. They may be single or multiple, and have been observed of a weight as high as 780 grams. *Perineal calculi* resemble urethral calculi, and are formed in the perineal tissues under conditions of urethral obstruction or trauma similar to those giving rise to the latter.

Preputial calculi are formed beneath the prepuce in cases of phimosis, and are composed of fatty material, fatty acids, cholesterol, or epithelium, derived from inspissation of the smegma and impregnation with phosphates. They may be single or multiple; in one case two stones had a combined weight of 42 grams.

Prostatic calculi are often found in the prostate of adults and old men, and are occasionally discharged with the urine. The glandular alveoli of the prostate normally contain numerous minute muco-calculous, round, concentrically marked bodies, which by accretion of calcareous and phosphatic material may attain macroscopic dimensions. They are ordinarily multiple. In one case the combined mass of calculi weighed 105 grams; in another case a single concretion weighed 25 grams. Calcareous concretions have also been found in the *seminal vesicles*.

Calculi rarely occur in the *oviduct*, *uterus*, and *vagina*. In the oviduct calcium-oxalate calculi have been found. In the uterus and vagina they are usually calcareous, originating from vesical calculi passed through fistulæ, in vaginal cysts, as an incrustation on hairpins, pessaries, or foreign bodies in the vagina, or otherwise. Calculi obstructing the *nipple* have been observed.

In gout the deposits of urates in the *articular tissues* or joint cavities and elsewhere may assume a calculous character (chalk stones, tophi).

In the *brain* or its meninges concretions are represented by the rare psammomata or tumors containing calcareous particles, also by calcified necrotic areas or tubercles. Calcareous concretions or granules (psammoma) have been noted in the auditory, facial, and optic *nerves*, especially the first-named, and also in the inner ear.

Dacryoliths, or lachrymal calculi, have been observed in the lachrymal gland, the conjunctiva of the upper lid, the ducts of the Meibomian glands, the conjunctival cul-de-sac, the lower canaliculi, and the lachrymal passages. They consist of the usual calcareous and phosphatic ingredients, and in the lachrymal passages frequently have a nucleus of leptothrix fungi.

Characters and Tests of Calculi.

In the examination of calculi, they should be sawed or broken in two to observe if they are uniform or heterogeneous in structure. In the former case the sawdust may be used for analysis, in the latter fragments from the different portions may be powdered. The pulverized material is employed in testing as to solubility, the effect of heat, etc. In applying heat, a small portion of the powder is placed on platinum foil and heated to a

red or white heat in the Bunsen or alcohol flame. For certain purposes the blowpipe flame is used.

Phosphatic calculi, composed of either calcium phosphate or ammonio-magnesian phosphate alone, or of both mixed, or mixed with other substances, are of the most general occurrence in the body. They are usually white or gray in color, with either a smooth or rough surface, rather soft, friable, and easily crushed, and have a stony fracture. Adherent or embedded crystals of ammonio-magnesian phosphate may appear as glistening points. The powdered material of which they are composed is insoluble in water and alkalis, readily soluble without effervescence in water acidulated with acetic or hydrochloric acid, and is precipitated from this solution on making it alkaline with soda, potash, or ammonia. When heated on platinum foil to a red heat the powder does not melt, char, or burn, and suffers little or no diminution in amount. Ammonio-magnesian phosphate melts under the blowpipe flame, while calcium phosphate does not. On mixing powdered ammonio-magnesian phosphate with calcium or potassium hydrate and moistening, ammonia is evolved (especially if heated), and may be recognized by its odor and its turning moistened red litmus paper, suspended near, to a blue color.

Calcium-carbonate Calculi.—Calcium carbonate is frequently present in calculi mixed with calcareous or phosphatic material, but rarely is the sole or predominating constituent. When pure, the calculi are gray, smooth, and hard. On being touched with acid effervescence occurs; on heating to a white heat the powder first turns black and then white again, calcium oxide being formed, which dissolves in water sparingly and in that solution gives an alkaline reaction.

Cholesterin Calculi.—Cholesterin forms the bulk of most biliary calculi, or may constitute practically their entire substance; it is frequently present in calculi from various parts of the body, especially in those derived largely from fatty material, as seboliths and preputial concretions; one or two instances of cholesterin urinary calculi are known. The color of cholesterin biliary calculi may be modified by the presence of bile pigment, and their consistency by the presence of calcareous salts. When in a state of approximate purity cholesterin concretions are white or gray, smooth, of waxy consistency, soft and easily crushed, and float

in water. Cholesterin melts at 137° C. and at a little higher temperature burns with a yellow carbonaceous flame without leaving any residue. It is soluble in ether, chloroform, and hot alcohol. The addition of a drop of strong sulphuric acid to the chloroform solution of cholesterin produces a deep-red color. On allowing a drop of the hot alcoholic solution to cool on a slide the characteristic rhombic colorless plates, frequently with a corner lacking or with a side of an echelon or step-ladder form, crystallize out and may be recognized with the microscope.

Bile-pigment Calculi.—Bile pigments (bilirubin, biliverdin, etc.) enter into the formation of most biliary calculi to a greater or less extent, giving them a brown, dark-green, or yellow color. Occasionally biliary calculi are composed chiefly or entirely of bile pigment. Such calculi are hard, non-crystalline, sink in water, and yield the characteristic color reactions with nitrososulphuric acid; when heated they do not melt or burn, but char and leave an ash. Bilirubin may be extracted with chloroform, in which the other bile pigments are insoluble.

Fatty Calculi.—Fatty material, in the form of neutral fat, soaps, or fatty acids, occurs in many calculi in which there has been an inspissation of organic matter, as in seboliths and preputial concretions. Fat or fatty acids may enter into the formation of biliary calculi, and rarely form the preponderating ingredient. Four or five cases are known of urostealiths, or urinary calculi composed of fatty or soapy material, sometimes mixed or covered with phosphates, and in two or three cases similar concretions have occurred in the stomach and bronchi. Urostealiths are brown or yellow, soft and friable when fresh, hard and brittle when dry, softer again when warmed. The material is soluble in ether, saponifies with caustic alkalies; when heated it burns with a yellow flame and an odor of resin or shellac, and is entirely consumed.

Uric-acid calculi are formed in the kidney and urinary organs. They are hard and brittle, concentrically laminated, brown or reddish, with smooth or tuberculated surfaces, and vary in size from small particles up to a weight of 150 grams. The powdered material yields the murexide reaction (page 191), is soluble in caustic alkali, and on being heated on platinum foil it is entirely or almost entirely consumed without flame, leaving only a trace of residue or none at all.

Urate calculi occur in the urinary organs and gouty joints. The gouty tophi consist of sodium urate. In the urinary calculi, the various urates, especially of ammonium, calcium, and sodium, may be present, usually associated with uric acid or calcium oxalate, though in children urates may form the predominating ingredient. Urate calculi are usually small, not over a couple of centimetres in diameter, of a light-yellow, brown, or gray color; they are not so hard and dense as the uric-acid stones. Urates all yield the murexide reaction, and are soluble in hot water.

Ammonium urate is completely consumed on heating. On mixing a portion of powdered ammonium urate with calcium or potassium hydrate and moistening, ammonia is evolved and may be recognized by its odor and by moist red litmus paper suspended over the mixture turning blue.

The other urates leave a residue after heating. Sodium and potassium urates melt and leave a residue of carbonate which is alkaline when moistened; sodium urate imparts the characteristic yellow color of sodium to the Bunsen or alcohol flame. Calcium and magnesium urate do not melt.

Xanthin calculi have been found in the urine a few times. They are white to brown in color, of medium hardness, and range in size from that of a pea to that of a hen's egg. Uric acid may be mixed with the xanthin. The powder of xanthin calculi on being heated is entirely consumed, without flame. Xanthin does not yield the murexide reaction; on dissolving a minute portion in a drop of nitric acid and carefully evaporating, the residue turns bright-yellow and is insoluble in potassium-carbonate solution, but is soluble in potassium-hydrate solution with the formation of a reddish color.

Calcium-oxalate calculi, nearly pure or mixed with uric acid or urates, are among the common urinary calculi, and have been also found in the female genitals. They are very hard and dense, dark-colored; the larger forms have a rough, tuberculated surface ("mulberry calculi"); sometimes they form small, round, seed-like bodies. On being heated, the powder first chars, and then becomes converted to a white residue, calcium carbonate, which effervesces with acid; on still further heating the calcium carbonate is changed to calcium oxide, which affords an alkaline reaction when moistened.

Indigo calculi, found in the kidney in three or four cases, have a dark-brown or blue-black color, and when drawn over paper leave a blue mark. On being heated a sooty odor is evolved, and the material sublimes; on condensing the vapor on a glass slide and adding glycerin, blue prismatic crystals and granules are seen. The solution in sulphuric acid is brownish and then muddy blue; on diluting and filtering a clear blue fluid is obtained.

Cystin calculi, rarely found in the bladder, are usually of medium size, with finely granular or crystalline surface, rather soft, and of a pale-yellow or gray color. Cystin is soluble in ammonia, and on evaporation the characteristic hexagonal crystals are deposited. It is also soluble in soda, potash, and mineral (not vegetable) acids. On being heated, cystin burns with a blue flame, evolves a sulphurous odor, and is entirely consumed without yielding any residue. If dissolved in potassium-hydrate solution and boiled with lead acetate, a black precipitate of lead sulphate is formed.

Calculi composed of **animal matter**, as fibrin, on being heated burn with a yellow flame, yield an odor of burnt horn, and leave little or no residue. They are largely soluble in potassium-hydrate solution. Microscopical examination may show epithelium, bacteria, etc., and in the case of coproliths fragments of food residue.

XI. PARASITES.

The examination of parasites, animal and vegetable, frequently comes within the field of the clinical laboratory. The subject is an extensive one, and its mastery requires much special study and opportunities that are afforded to few. It is only the common parasitic forms with which the ordinary clinician has the opportunity to become familiar. Outside from common forms the subject can be only very briefly covered here. It should be remembered that of the animal and vegetable organisms found associated with the human body, only a portion are pathogenic and injurious, while others are adventitious, accidental, and innocuous.

ANIMAL PARASITES.

The animal parasites of man belong to the protozoa, the vermes, and the arthropoda.

Protozoa.—The *parasite of malaria* (page 33) occurring in the blood, and the *Amœba coli* (page 110), occurring in the colon in amœbic dysentery and in amœbic abscesses of the liver and lung, have been already considered. Other amœbæ have at times been observed in various fluids and parts of the body.

Psorosperms and Coccidia.—These parasitic protozoa are common in rabbits, very rare in man. They are oval forms 20 to 40 μ long (*Coccidium oviforme*). At first the organism is granular throughout; at a later stage of development the granular substance collects in a ball in the centre of the cell, and still later it breaks up into four spores. In man the coccidia develop most often in the epithelium of the intestine or bile passages of the liver, causing intestinal erosions or hepatic neoplasms. They have also been observed in the human kidney, wall of the ureter, and in empyemic pus, and are perhaps associated with certain skin lesions (as Paget's disease, Darier's disease).

Related protozoa (*balbiana*) have in one or two instances been found encysted in human muscle. Protozoan forms have been claimed to cause cancer, but the subject is not yet settled.

Cercomonas.—This is a pear-shaped organism about $10\ \mu$ long, coming to a filamentary point at one end and with a flagellum at the blunt end. It has been found in the intestine in diarrhoeal conditions; also in the liver and mouth.

Trichomonas Vaginalis.—This is a pear-shaped parasite 15 to $30\ \mu$ long with three or four flagella on the blunt end and a longitudinal undulating membrane or comb of cilia. It is most frequently found in the vagina, where it is innocuous; it has also been observed in the bladder, associated with hæmaturia, and a similar parasite has been found in the intestine and mouth.

Megastoma Entericum.—This is a pyriform organism found a few times in the human intestine, 10 to $20\ \mu$ long, with an oral depression at the blunt end, and four pairs of flagella.

Balantidium coli is an oval, asymmetrical body, 70 to $100\ \mu$ long, with an oral and an anal structure, an elliptical nucleus, and two contractile vesicles. The surface is entirely covered with cilia. In Scandinavia it is common in the intestine, especially in diarrhoeal conditions, rarer in other countries.

Vermes.—The worms parasitic in man belong to the classes of trematodes or flukes, cestodes or tapeworms, and nematodes or round worms.

Trematodes, or flukes.—These are mostly flat, fish-shaped worms infesting various parts of the body. Only two or three forms are common in man, in certain countries, the others being rare in man and all rare in America.

Schistosoma Hæmatobium.—This is a bisexual fluke, 4 to 20 millimetres long, occurring chiefly in the veins of the pelvic viscera, bladder, rectum, and vagina. By the accumulation of the ova in the capillaries, their irritative effects, and rupture of the vessels into the neighboring cavities, marked local disturbances are produced, as local inflammations, ulcerations, nodular growths, hemorrhages (vesical, rectal, vaginal, etc.). The ova may also pass into other parts of the portal system, as the mesenteric glands and liver, and have even been found in metastatic abscesses in the lungs. The ova and perhaps embryos occur, along with blood, in the urine, fæces, vaginal discharges, or pus of metastatic abscesses. The worm has been known to develop in the lungs, discharging ova into the sputum. *Schistosoma* infection is common in Egypt, and also occurs in South Africa and

elsewhere; only two cases have been reported in the United States, both originating in Africa.

Paragonimus Westermanii.—This fluke is a common parasite of the lung of man in eastern Asia, causing hæmoptysis and other symptoms. It occurs rarely in the brain, liver, intestinal walls, and elsewhere. The ova appear in the sputum, and in the rare intestinal cases in the fæces.

Fasciola Hepatica, or liver fluke.—This is common in the liver of animals, but is very rarely found in the liver of man, exceptionally in the lungs or elsewhere. The ova appear in the fæces. **Dicrocoelium lanceatum** has been reported in the human liver about six times. **Opisthorchis felineus** and **Opisthorchis sinensis** cause human liver disease in Asia. **Distoma conjunctum** has also been found in the liver. **Fasciolopsis Buskii**, **Heterophyes heterophyes**, and **Amphistoma hominis** have been observed in the human intestine. **Monostomulum lentis** and **Agamodistomum ophthalmobium** have been found in the eye.

Cestodes, or tapeworms.—These are parasitic under two conditions, namely, as adult forms or strobilæ occupying the intestine, and as larval forms developing in the solid viscera or tissues of the body. Most tapeworms found in man occur only in the adult form; two (*Tænia echinococcus* and *Sparganum Mansonii*) occur only in the larval stage; one (*Tænia solium*) occurs in both adult and larval stage. The following cestodes have been found in man, in the stage mentioned:

Tænia saginata: adult form.

Tænia solium: adult form in intestine; larval form (*cysticercus cellulosæ*) in muscles, brain, eye, and elsewhere.

Tænia echinococcus: larval form (*hydatids*) in liver, lungs, and elsewhere.

Tænia confusa: adult form.

Hymenolepis murina: adult form.

Hymenolepis diminuta: adult form.

Dipylidium caninum: adult form.

Davainea madagascariensis: adult form.

Diplogonoporus grandis: adult form.

Dibothriocephalus latus: adult form.

Dibothriocephalus cordatus: adult form.

Sparganum Mansonii: larval form in subperitoneal tissue.

Of these only the *Tænia saginata* (page 111) is common in

this country. The other forms occur rarely or in distant countries.

Nematodes, or round worms.—These comprise a numerous and important class of parasites, of which the following are the chief well-established species found in man:

Ascaris lumbricoides, common in the intestine, and occasionally found in adjacent cavities (page 112).

Ascaris canis, occasionally found in the human intestine.

Ascaris maritima, once found in the human intestine.

Oxyuris vermicularis, a small, thread-like worm, common in the intestine (page 112).

Diectophyme renale (*eustrongylus gigas*) is a very large worm, 30 to 100 centimetres long, which in man has been found in the pelvis of the kidney in a few cases. The ova may appear in the urine.

Strongylus longevaginatus has been found in the human lungs and stomach in two or three instances.

Uncinaria duodenalis (*Ankylostoma duodenale*) is an intestinal parasite causing extreme anæmia (page 112).

Strongyloides intestinalis is an intestinal parasite causing diarrhoea (page 113).

Trichuris trichiura (*Trichocephalus dispar*) is a common but innocuous intestinal parasite (page 113).

Trichinella Spiralis.—The adult forms of this worm, 1.5 to 3 millimetres long, occur in the intestine (page 113), where large numbers of living embryos are given off. These embryos make their way through the intestinal walls into the muscles of the body generally, where they form cysts (about .5 millimetre long) and produce severe disturbance. In the early stage of trichinosis the worms may be found in the fæces; later the encysted trichinæ may be found in portions of the muscle (preferably from the deltoid or biceps) removed for examination.

Filaria Sanguinis Hominis.—The various forms of the filariæ of the blood, namely, *Filaria Bancrofti* and its larval form *Filaria nocturna*, and the larval forms *Filaria diurna*, *perstans*, and *Demarquaii*, have been considered in connection with the blood (page 40).

Filaria loa is not uncommon in Western Africa, occurring in the subcutaneous and subconjunctival tissue; the *Filaria diurna* occurring in the blood is probably its larval form.

Other forms of filariæ have in isolated cases been noted in the human eye, mouth, respiratory passages, and elsewhere, as *Filaria oculi*, *Filaria labialis*, *Filaria hominis oris*, *Filaria bronchialis*, *Filaria testiformis*, *Filaria lymphatica*, *Filaria volvulus*, *Filaria inermis*, *Filaria Magalhæsi*, etc.

Dracunculus medinensis, or Guinea worm, is a slender nematode sometimes reaching nearly a metre in length, that frequently produces subcutaneous abscesses in the tropical regions of Asia and Africa.

Rhabditis Niellyi is an immature nematode allied to filaria observed in one case in cutaneous vesicles and the blood.

Rhabditis pellio is a small nematode about a millimetre long that has been found in the vagina and urine in two or three cases.

Arthropoda.—The arthropoda parasitic in man belong to the classes arachnida, myriapoda, and insecta. These parasites inflict bites or stings; inhabit the clothing, the surface, or the superficial cavities of the body; burrow into and beneath the skin; or deposit their ova in the tissues or the superficial body cavities, where the larvæ then develop.

Arachnida, or spiders.—The most representative forms are as follows:

Pentastoma tænioides is a mite common in the nasal cavity of dogs. It is occasionally found in the adult condition in the nasal fossa of man, where it occasions inflammation; its larval form (*Pentastoma denticulatum*) has been often found in the human liver, less often in the lungs, kidney, spleen, and elsewhere, where it may not cause much trouble. An allied larval form, *Pentastoma constrictum*, has been seen in the human liver, lungs, and intestinal mucosa.

Demodex folliculorum is common in the sebaceous glands about the face, and occasions little or no trouble.

Sarcoptes scabiei, the itch mite, burrows under the skin, where it deposits the ova, and produces scabies. Other species of scabies infecting man are known.

The ticks (*ixodes*, *argas*) and the mites (*leptus*), including the chigger of the middle United States, attach themselves to or burrow into the skin, producing irritable sores. Some of the higher spiders and scorpions produce poisonous bites, rarely severe or dangerous.

Myriapoda.—The centipedes may inflict poisonous bites, sometimes severe or dangerous. Myriapoda are alleged to be capable of causing trouble by finding their way into the ear, nose, etc.

Insecta.—Many varieties of insects are capable of inflicting painful and poisonous bites and stings, especially among the heteroptera, diptera, and hymenoptera. The pediculi (lice), cimex (bedbug), culicidæ (mosquitoes), aphaniptera (fleas), are well-known pests which irritate by their superficial bites.

In another class of cases marked and even dangerous lesions are produced by the deposition of ova in the tissues or body cavities, and their subsequent development to the larval stage (maggots, etc.). In this manner the œstrus, flies (*muscæ*), and especially the *Lucilia macillaria* or screw-worm of the Southern States, are productive of abscesses, ulcers, inflammation, destruction of tissues, etc., especially in the nose or subcutaneous tissue.

VEGETABLE PARASITES.

The vegetable parasites of man are comprised in two broad classes, the Fungi and the Bacteria. The bacteria are of pre-eminent clinical importance, and the methods of their investigation are briefly presented in the following chapter.

Fungi.—The fungous parasites of man are much less common than the bacteria, though some of them produce serious lesions. Some of them distinctly produce lesions (pathogenic parasites), while others grow adventitiously in the tissues secondary to other conditions without themselves causing any trouble (saprophytic parasites). The growth of fungi in the tissues is often very different from their growth on media. Many of the pathogenic fungi, as the actinomyces and *Oidium albicans*, have not been definitely classified in the botanical system.

Fungous growths in general consist of a vegetative portion, the mycelium, and a reproductive or spore-forming portion. The mycelium consists of a growth of fine filaments, interlacing, branching, or variously arranged. The spores are produced sometimes within the mycelial segments, sometimes in or on specialized structures. In many species and under propitious conditions sexual spore-formation occurs from specially developed structures.

Fungous growths are examined and determined in the fresh condition, after staining, by cultivation on media, and by animal

inoculation. In fresh condition they may be examined by teasing them out in water or glycerin, by clearing away the adjacent material with liquor potassæ, etc. They may be stained by the methods employed for bacteria, as Gram's stain, carbol-fuchsin, etc.; many of them do not stain well. They may be cultivated on the ordinary bacterial media, as gelatin or agar, on moist sterilized bread, potato, etc., or on special media; many fungi grow better on acid than on alkaline media.

The chief varieties of fungi parasitic for man are as follows:

Aspergillus.—Several species of this mould are parasitic and pathogenic for man, namely, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus nidulans*. *Aspergillus fumigatus* is the commonest and most pathogenic, as it grows not only on free surfaces but is capable of producing serious lesions of the internal viscera, while the others grow chiefly only on free surfaces. The commonest seat of growth of these fungi is the external auditory canal (otomycosis), where they form pseudo-membranes or plugs; they have also been met with in the antrum of Highmore, the cornea, the anus, in the lungs as secondary growths in tuberculosis and other wasting diseases, and elsewhere. In several reported cases *Aspergillus fumigatus* has caused primary pseudo-tubercular lesions in the lungs, and when its spores are injected into susceptible animals it is capable of producing disseminated and rapidly fatal lesions in the liver, kidney, lungs, and other viscera. The growth of these fungi in the ear consists of a luxuriant proliferation of mycelium, sporangia, and spores; in the lungs and viscera the *Aspergillus fumigatus* grows chiefly as a mycelial network, rarely in ray form or with a tendency to sporulation.

The mycelium of *aspergillus* consists of a network of branching segmented filaments. The spores are developed on spherical, oval, or club-shaped bodies borne on a shaft or stalk projecting from the mycelium. From the surface of this spore-bearing head project minute branches (sterigmata), on which the spores are borne. Sclerotia, or dense rounded masses, are sometimes produced.

Aspergillus fumigatus: The growth is greenish, bluish, or grayish. The mycelial filaments are 2 or 3 μ in diameter. The spore-bearing branches are short, with a shaft 5 μ thick, a club-shaped head 8 to 20 μ in diameter, bearing short unbranched

projections or sterigmata about $6\ \mu$ long, which are studded with smooth, rounded spores 3 to $4\ \mu$ in size. Sclerotia not observed.

Aspergillus flavus: The growth is yellow or greenish. The spore-bearing head is borne on a shaft 4 millimetres long and 7 to $10\ \mu$ thick. The sterigmata are unbranched, often developed only on the upper half of the head. The spores are round, 5 to $7\ \mu$ in size. The sclerotia are minute.

Aspergillus niger: The growth is dark-brown. The spore-bearing head is spherical, up to $75\ \mu$ in diameter, borne on a stalk up to 8 millimetres long and 10 to $15\ \mu$ in diameter. The sterigmata are 20 to $100\ \mu$ long, branched at their extremities. The spores are very numerous, round, 3.5 to $5\ \mu$ in size. The sclerotia are about 1 millimetre in diameter.

Eurotium glaucum and *Eurotium repens* are forms closely related to *aspergillus* that have been found in some cases of otomycosis. They differ from *aspergillus* chiefly in their sexual sporulation structures, exhibiting perithecia instead of sclerotia.

Mucor.—This genus somewhat resembles *aspergillus* macroscopically and in its parasitic and pathogenic properties. The mycelial threads are little or not at all segmented. The sporangia or spore-bearing organs are rounded vesicles on a stalk; within the vesicles is an irregular mass of spores. A number of species parasitic in man have been observed, as *Mucor corymbifer*, *Mucor septatus*, the related *Rhizomucor parasiticus*, and other uncertain species. These fungi grow in the auditory canal, and have been observed a number of times in the lungs, usually in association with exhausting diseases like carcinoma. In one case a *mucor* caused a disseminated and fatal infection, producing abscesses, ulcers, and other lesions in the pharynx, intestine, larynx, lung, and brain. The growth of *mucor* in the tissues may consist of both mycelium and spores.

Verticillium Graphii and *Penicillium minimum*, forms somewhat similar to *aspergillus*, have been observed in cases of otomycosis.

The various forms of tinea or ringworm are caused by two or three distinct kinds of fungi, the *Microsporon Audouini*, *Trichophyton megalosporon endothrix*, and *Trichophyton megalosporon ectothrix*, beside occasional atypical related forms. To examine ringworm lesions for the fungi take a portion of the affected hair shaft or root, or scrape off a piece of the affected epidermis (previously if necessary macerated with a little liquor

potassæ), and place it under the cover glass with a 5- to 20-per-cent solution of potassium hydrate; examine with the microscope during and after the process of clearing. The fungi are easily grown on media. The ordinary staining methods (as Gram's) are not very satisfactory.

Microsporon Audouini causes most of the cases of tinea tonsurans in children, and may produce lesions on the non-hairy skin differing from typical tinea circinata; it does not affect the beard or nails or occur in adults. The growth on the hair consists of mycelium and spores. The spores are closely packed so as to form a layer on the outer surface of the hair, beginning just above the bulb and extending upward; they are round, with a double contour, and 2 to 3 μ in diameter. The mycelial threads, about 2 μ in diameter, slightly branching, are situated longitudinally in the peripheral portion of the hair beneath the layer of spores. Just above the bulb the mycelium threads form a characteristic fringe projecting downward a little beyond the spores. The mycelial threads are divided into (usually) rather long segments, each containing dark spots.

Trichophyton megalosporon endothrix causes a considerable proportion of the cases of tinea tonsurans and tinea circinata; it does not affect the beard or nails. It grows in the *interior* of the hair, in the form of longitudinal chains or rows of spores, sometimes massed together so as to obscure the chain formation. The spores are quadrangular, oval, or rounded, 5 to 7 μ in size. Mycelium is not conspicuous, other than as longitudinal filaments breaking up into chains of spores.

Trichophyton megalosporon ectothrix, derived from animals, is the cause of tinea sycosis, onychomycosis, a large proportion of cases of tinea circinata, and a few of those of tinea tonsurans. It may produce suppurative lesions. It grows chiefly as a sheath on the *exterior* of the hair root, perhaps invading the hair substance to a slight extent (endo-ectothrix). Like the endothrix, it grows in longitudinal chains of spores 4 to 12 μ in diameter.

Achorion Schoenleinii.—This is the fungus of favus, and has also been observed in the gastro-enteric tract. The crust of favus is composed almost entirely of this fungus, while the affected hairs may be extensively infiltrated with it. To examine it, place a portion of the crust or affected hairs or epidermis on a slide and cover it with a drop or two of liquor potassæ;

after a few minutes the fungus is ready for examination, when it may be covered with a cover-glass, or the potash solution may be removed with bibulous paper and replaced with glycerin.

The fungus consists of a tangled network of branching mycelial threads mingled with large numbers of spores, all of a grayish or pale-greenish color. The mycelial threads are from 2 to 3 μ in calibre; some of them are simple and empty, others are divided into short segments and exhibit all stages of spore-formation. The spores vary in size from 2.3 to 5 μ , and also vary in shape, being round, oval, kidney-shaped, pyriform, dumb-bell form, etc. Several varieties of this species have been reported but not definitely established.

Microsporon furfur grows in the horny layer of the epidermis, causing tinea versicolor. It appears as a luxuriant growth of spores, some single, many aggregated in clusters, in the midst of a mycelial network. The spores vary in size and shape, being round or oval, 3 to 8 μ in size, very refractile. The mycelial threads are 1.5 to 4 μ in diameter, straight or curved, and contain spores in places. To examine for the fungus scales scraped from the macules are placed under a cover-glass with liquor potassæ.

Microsporon minutissimum is the fungus of the skin disease erythrasma. It consists of a network of very fine mycelial threads divided into short segments, along with granular material. It is possibly a bacterium.

Oidium Albicans.—The botanical place of this fungus is still uncertain, although numerous names and places have been assigned to it. It grows most frequently on the mucous membrane of the mouth, forming the whitish membranes or aphthæ of thrush. It also grows occasionally in the pharynx, œsophagus, vagina, vulva, and prepuce; it has been observed on the nipples of mothers nursing infected infants, and has in a very few cases been reported in the stomach, intestine, respiratory passages, and lungs. It thus grows by predilection on mucous surfaces lined with squamous epithelium. Zenker reported its occurrence in the brain.

The growth consists of segmented mycelial threads 2 to 6 μ in diameter, slightly granular, mingled with an abundance of clear, rounded or oval, spore-like (and also yeast-like) bodies 6 to 10 μ in size. These rounded bodies are partly free, partly grow

interposed between the long mycelial segments, partly grow out laterally from these segments in the form of buds or clusters.

Actinomyces (ray fungus).—This fungus produces abscesses, chronic inflammation, ulcers, tubercular growths, etc., in the parts infected; the lesions may occur in various parts of the body, especially the head and neck, tongue, lungs, abdominal viscera. The growth forms minute round, yellow granules, which are very characteristic of the pus or lesions. The centre of these bodies when fully developed consists of mingled mycelial filaments, minute spores, and granular material; outside this is a zone of filaments arranged radially; and next outside this are radiating club-shaped bodies, with their large ends outward; the outermost portion consists of inflammatory cells.

The fungus can be examined by crushing the granules under a cover-glass, or by hardening and sectioning the tissues affected. Specimens examined fresh show the clubs well, but not the mycelium. Addition of strong hydrochloric acid or liquor potassæ, with the cover-glass then applied, shows the fungus well. Hardening in Müller's fluid brings out the structure without further preparation. The parts of the fungus can also be demonstrated by staining with hæmatoxylin and eosin, or by the ordinary bacterial stains. Gram's method shows the mycelium well.

There are other fungous parasites and species closely related to ordinary actinomyces, among which is probably that causing Madura foot or mycetoma.

Streptothrix.—This group embraces the "branching bacteria" and the actinomycetes. In a few instances pathogenic branching bacteria have been found, causing visceral abscesses, pseudo-tubercular lesions of the lungs, and other conditions.

Saccharomycetes, blastomycetes, or budding fungi.—In this group the cellular, spore-like elements multiply by the growth of buds.

The commonest and best-known representative of this group is the yeast fungus, or *Saccharomyces cerevisiæ* (Fig. 17). This consists of oval bodies, variable in size, 3 to 10 μ long, occurring singly or with two, three, or four united together in chains. They multiply by budding, and buds may be seen projecting from parent cells in all stages of development, from minute out-growths to a size equal to that of the parent. This organism has

the power of producing the alcoholic fermentation of sugar. Yeast fungi are usually present in the stomach contents (at least after bread diet), frequently in the fæces, sometimes in the urine (especially in diabetics), producing no other evil effects than occasional fermentation.

Fungi of the *saccharomyces* type (blastomycetes) have been found parasitic and pathogenic in a few cases, causing chronic inflammation and ulceration of the skin, endometritis, and abscesses and other lesions in the bones and viscera. In cultures of these pathogenic yeasts on media mycelial threads may appear as well as the oval bodies. In the affected tissues only the budding oval forms are seen; they stand out conspicuously on treatment of unstained sections with liquor potassæ.

Some of the parasites reported in cancer are of the *saccharomyces* type, but their etiological relationship is not established.

Leptothrix.—This is commonly classed among the bacteria and consists of a luxuriant growth of large, rod-like segments or bacilli growing end to end in long filaments. It is very frequently found in the mouth (*Leptothrix buccalis*), also in other situations, and has little or no pathogenic power. In one case of puerperal septicæmia *leptothrix* was said to have been found in the blood during life.

XII. CLINICAL BACTERIOLOGY.

The procedures employed in the determination of the presence and kind of bacteria in morbid conditions may be broadly classed as cultural methods, microscopical methods, and animal inoculation.

Sterilization.—Methods of sterilization lie at the basis of bacteriological investigation. Sterilization may be accomplished by means of dry heat, moist heat (boiling, steam, superheated steam), chemical antiseptics, and filtration through porcelain.



FIG. 35.—Hot-air Oven for Sterilization. (Lentz & Sons.)

Dry heat: Metal instruments may be sterilized by heating them a few moments in a Bunsen or alcohol flame; this injures steel, but for certain purposes it is convenient to have an old pair of scissors, knives, etc., available for quick sterilization in this way. The platinum wire and loop are sterilized by heating the wire to a white heat, and then passing the glass handle a few times to and fro in the flame; platinum suffers no injury by this treatment. Glassware, as test tubes and Petri plates, may be

sterilized by heating in a hot-air oven, a large sheet-iron oven heated by a large gas flame beneath (Fig. 35), at a temperature of from 150° to 200° C.

Boiling in water (or 1-per-cent sodium-carbonate solution) for five to fifteen minutes is a useful means of sterilizing instruments or glassware. Water and most solutions may be sterilized by boiling.

Sterilization by *steam* is applicable to instruments, glassware, culture media, etc. Arnold's steam sterilizer is one of the most convenient for the purpose (Fig. 36). The objects to be sterilized are placed in the steam chamber for one-half to one hour or more while the steam is generated from below; the temperature thus applied is slightly over 100° C., at the ordinary atmospheric pressure.

Discontinuous sterilization is a method employed for culture media. The material is heated in the steam sterilizer once each day for three successive days, fifteen to thirty minutes each time. Between the sterilizations the culture medium is kept at a temperature of 20° to 37° C. in order to encourage the development of spores resistant to heat.

Sterilization by *steam under pressure*, or superheated, is accomplished by an apparatus called the autoclav; one exposure of fifteen minutes under a pressure of one extra atmosphere (giving a temperature of 122° C.), is sufficient. Except that sterilization is completed in less time, the method offers no great advantages for laboratory purposes over the ordinary cheaper steam sterilizer.

Antiseptics: Mercury bichloride in .1-per-cent solution is a useful antiseptic for the skin, glassware, or for saturating cloths used for temporarily wrapping organs from autopsy. Phenol in 2- to 5-per-cent solution is useful for sterilizing instruments, syringes, etc. Traces of chemical antiseptics may, if desired,

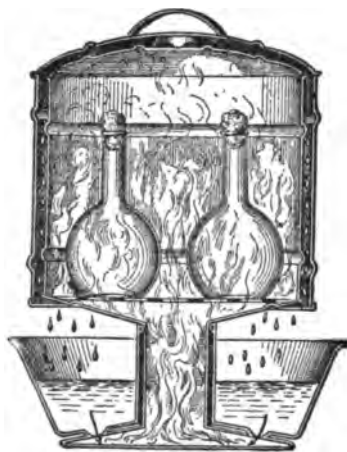


FIG. 36.—Arnold Steam Sterilizer. (Willmot Castle & Company.)

be removed after sterilization by rinsing with clean sterile water.

When it is desired to study the products of bacterial growth without the alterations which the use of heat or chemicals would produce, the bacteria may be removed by *filtering* the fluid containing the growth through a porous, unglazed porcelain filter, as Chamberland's filter.

A. CULTURAL METHODS.

Culture Media.—Artificial media of various kinds, fluid and solid, are employed for observing the mode of growth and chemical products of bacteria. The chief culture media employed are bouillon, gelatin, agar, blood serum, milk, potato.

Preparation of Culture Media.—These media in general are put up in test tubes in which they are safe against contamination. The best size for the tubes is about 15 by 1.8 centimetres.

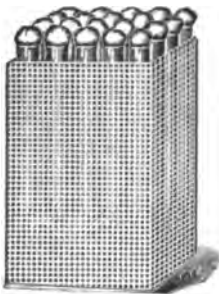


FIG. 37.—Wire Basket for Culture Tubes. (Bausch & Lomb.)

The tubes should be thoroughly cleaned with soap and water, or with the bichromate solution (page 5) if necessary. Old tubes containing material once used are first boiled to destroy the germs and dissolve out the media. Into the mouth of each tube is inserted a plug of non-absorbent cotton two or three centimetres long; these plugs (unless wet) prevent the entrance of bacteria or spores of moulds, and it is important that they be kept dry and unmixed with the culture media. It is cus-

tomary to sterilize the empty tubes, thus plugged, before introducing the culture media, either in the hot-air oven or steam sterilizer; this preliminary sterilization may, however, be dispensed with. For convenience in handling large numbers of tubes they are placed upright in wire baskets (Fig. 37), a layer of cotton in the bottom of which prevents breakage.

Introduction of the various culture media into the empty tubes is much facilitated and expedited by the following arrangement: One end of a short rubber tube is attached to a funnel and into the other end is inserted a short glass tube or nozzle drawn to a point. A pinchcock on the rubber tube enables it to be opened or closed at will. The funnel is placed upright in

a support and into it is poured a quantity of liquid or liquefied culture media. The cotton plugs are removed from the tubes, one by one, the tube is held beneath the funnel, the pinchcock is held open long enough to allow 5 to 10 cubic centimetres of the medium to flow into the tube, and the cotton plugs are then replaced.

After depositing the requisite amount of culture media in the duly plugged tubes, they are sterilized, either in the ordinary steam sterilizer one-half hour each day for three days, or in the autoclav.

Culture media if kept a long time before use are apt to dry out and become useless and wasted. This may be partly obviated by placing air-tight rubber caps (sold for the purpose) or tying oiled silk over the ends of the tubes. Or a quantity of the media may be kept in bulk in flasks, kept closed air-tight by oiled silk; from these flasks tubes may be filled at intervals as required.

Bouillon.—This is a fluid culture medium, useful for many purposes. By the introduction of special substances it is employed for special purposes; and gelatin and agar media consist practically of bouillon to which enough gelatin or agar has been added to make the material solid at ordinary temperatures. Bouillon consists essentially of a watery infusion of the principles of lean meat; to which it is customary to add $\frac{1}{2}$ per cent of sodium chloride, 1 per cent of peptone, and sufficient alkali to make the fluid neutral or slightly alkaline. The best peptone for bacteriological purposes is Witte's dry peptone.

Meat bouillon : Bouillon prepared directly from meat is probably rather more nutritious and efficient than that made with meat extract, but is more troublesome to prepare. It is made as follows: 500 grams of lean beef or mutton free from tendon, fat, etc., is chopped fine and soaked in one litre of water in an ice box for twenty-four hours. At the end of that time all the liquid is pressed or squeezed out of the meat through a piece of muslin. In the fluid thus obtained 10 grams of peptone and 5 grams of pure sodium chloride are dissolved, and the whole is boiled in an enamelled iron pan until the albumin is entirely precipitated. During the boiling the fluid is carefully and exactly neutralized or made slightly alkaline by adding a small amount of a strong solution of sodium hydrate or sodium carbonate, until the proper reaction, as shown by litmus paper, is attained.

At the conclusion of the boiling, sufficient water is added to make the amount up to a litre, the fluid is filtered, introduced into the tubes (or a flask for storage), and sterilized in the steam sterilizer by the discontinuous method.

Meat-extract bouillon : The commercial meat extracts, as Liebig's or Armour's, are often used in the preparation of bouillon, being easier to use and for most purposes as efficient as the meat bouillon. About 3 grams of meat extract (Liebig's), 5 grams of sodium chloride, and 10 grams of peptone are boiled in a quantity of water, made neutral or slightly alkaline, water is added to make 1 litre, and the material then filtered, tubed, and sterilized.

Glucose-bouillon is the same as ordinary bouillon, with either one or two per cent of glucose added. This medium is used in testing the fermentative power of bacteria, and also where the added nutritive action of glucose is desirable.

Other sugars, as lactose, sucrose, may be used instead of glucose, to test fermentative action on them. A few drops of a strong aqueous solution of crude *litmus* may be added, coloring the bouillon blue, so that the formation of acid by the bacterial growth may be directly shown by reddening of the fluid. For some purposes slightly *acid* bouillon, or bouillon made to a definite alkaline or acid strength by titration with phenolphthalein, is used.

Gelatin.—This consists of bouillon to which 10 per cent of gelatin has been added. It is solid below about 25° C., liquid above that temperature; it cannot be used in the culture oven, and in summer cannot be easily managed. It is employed for isolating bacteria in plate cultures and for testing the liquefying action of bacteria.

Nutrient gelatin may be prepared with either meat bouillon or meat-extract bouillon. To a litre of the boiling bouillon containing .5 per cent of sodium chloride and 1 per cent of peptone, add 100 grams (per litre) of the best gelatin (as the French "gold-label" brand), and stir constantly until well dissolved. Neutralize in the usual manner, boil vigorously for ten to fifteen minutes (until albumin is entirely precipitated), and add water enough to make one litre.

If upon trial it is now found that the material will filter perfectly clear, it may be filtered without further treatment; but if it does not filter clear, it should be *clarified*, as follows: Cool the

liquid below 60° C. and mix with it an egg which has been thoroughly beaten with 50 or 100 cubic centimetres of water. Then boil the mixture for five or ten minutes, *without stirring*, so that the egg albumen will coagulate in large flakes and collect the fine suspended particles. The large coagula may be removed with a skimmer, but breaking them up into fine particles should be avoided. The material should after this second boiling be made up to a litre.

Filtration, the next step, presents some difficulties. Two or three thicknesses of filter-paper should be used, being folded into ridges to increase the filtering surface, or a wire filtering frame being employed. The paper is thoroughly moistened, and a portion of the melted gelatin poured in carefully or allowed to flow gently down a glass rod. As the fluid cools, filtration is apt to be retarded, but may be again promoted by adding another portion of the material hot and fresh from the flame. By thus adding the hot gelatin in successive portions filtration can be usually accomplished at the room temperature; but if difficulty is experienced, the filter, supported on a tripod with the flask containing the filtrate beneath, may be placed in the steam sterilizer until filtration is complete.

The gelatin is then introduced into tubes, sterilized in the steamer fifteen minutes daily for three days, and then set aside in an upright position to allow the gelatin to harden, a depth of about 5 centimetres being in each tube. Gelatin should not be heated longer than necessary, as excessive heating lowers its solidification point. After sterilization the gelatin media should remain clear.

As in the case of bouillon, special modifications of gelatin may be made, as the addition of 1 or 2 per cent of glucose, litmus, etc.

Agar.—This is one of the most useful bacteriological culture media, and is employed for a great variety of purposes. It consists of bouillon to which is added 1.5 per cent of agar-agar, an Oriental alga rich in vegetable gelatin. This culture medium solidifies at about 40° C.; it can therefore be employed for growing bacteria at body temperature, and bacteria can be introduced into it while melted without injuring their vitality.

To prepare a litre of nutrient agar, to about two litres of water add 3 grams of meat extract, 5 grams of sodium chloride,

10 grams of peptone, and 15 grams of agar-agar fibres cut into fine pieces; these ingredients are best added and dissolved separately. If meat bouillon is preferred, dilute the meat infusion about twice, salt, peptone, and agar being added in the same amounts. Bring the mixture to a boil in an enamelled iron pan, and boil it vigorously for half an hour or until the excess of water is evaporated. Stir the mixture until the ingredients are dissolved, and afterward neutralize in the usual manner. From time to time remove with a skimmer the scum that forms. Prolonged and vigorous boiling is necessary to obtain a medium clear of precipitate. It is well to have a mark on the side of the pan to show the level of one litre.

After boiling a sufficient time clarify the liquid with an egg, if necessary, in the same manner as with gelatin. The amount being brought by the addition of water to one litre, filter exactly as in the case of gelatin. Introduce into tubes, sterilize thirty minutes daily for three days, and after the third sterilization lay the tubes in an inclined position until the agar hardens in the form of a slant extending from the bottom of the tubes nearly to the cotton plugs. If a precipitate appears after sterilization the medium must be again melted, boiled, and filtered.

Glucose agar is the same as plain agar with 1 or 2 per cent of glucose added. Other sugars may be used instead, and *litmus* may be added. *Lactose-litmus-agar* (lactose 2 or 3 per cent) is sometimes used. *Glycerin agar* contains 6 per cent of glycerin, which is added after the agar is filtered, just before the material is tubed and sterilized.

Blood Serum.—The coagulated serum of the blood of cattle (and of the human subject when obtainable by venesection, etc.), affords a very nutritious culture medium, especially useful for certain bacteria that do not grow well on other media, and hence well adapted to post-mortem bacteriological examinations. It is thus prepared: At the slaughter-house a tall glass jar is filled with blood flowing from the freshly cut carotid artery. Rigid asepsis is unnecessary. The jar is set aside for a few minutes to allow firm clotting to take place, and it is then placed in an ice box for from twenty-four to forty-eight hours for the clot to contract and the serum to separate; during this period the jar should be handled or shaken as little as possible, to avoid disarranging the clot. The clear, straw-colored serum is then care-

fully decanted or drawn off with a pipette, and introduced into plugged test tubes, 5 to 10 cubic centimetres in each. The tubes are then placed in a hot-air oven or special coagulating oven in an inclined position, so that the serum forms a slanting surface from the bottom to the upper part of the tube. The temperature of the oven is brought up to about 90° C. and maintained there until the serum in the tubes is firmly coagulated throughout; constant attention will be required to see that the temperature does not reach the boiling-point, since in that case the serum will bubble and its surface when coagulated will be rough and broken instead of smooth. After being thoroughly hardened, the tubes may be placed upright, and are sterilized in the steam sterilizer in the usual way. The application of tight rubber caps until the tubes are used will prevent evaporation and preserve them longer in good condition.

Loeffler's blood serum, for the cultivation of the diphtheria bacillus, is prepared by mixing 3 parts of beef-blood serum with 1 part of glucose (1 per cent) bouillon. The mixture is tubed, coagulated, and sterilized as is plain blood serum.

Milk is used chiefly to test the acid-forming and casein-coagulating properties of bacteria. As usually employed litmus is added to reveal directly the reaction. To fresh milk, with the cream removed, is added a small amount of strong aqueous litmus solution to color it light blue; it is then placed in test tubes, a few cubic centimetres in each, and sterilized by the discontinuous method.

Potato is sometimes employed as a culture medium to aid in differentiating certain bacteria. The potatoes are washed and pared; cylindrical pieces of sufficient size to fit into the test tubes are cut out or punched out, and each cylinder is divided into two parts by a longitudinal oblique cut; one part is placed in each tube, so that a surface slanting upward is presented. The piece of potato should be raised slightly above the bottom of the test tube to leave a space for surplus water to collect; this may be accomplished by placing a short piece of glass rod or tube in the bottom of the tube to hold up the potato, or by the use of special tubes with a constriction near the lower end.

Inoculating Culture Media.—For inoculating or planting upon the culture media the bacteria desired to be grown, the “plati-

num needle" and the "platinum loop" are used (Fig. 38). These consist of a piece of platinum wire attached to one end of a glass rod as a handle. The glass rod serving as a handle should be about 6 millimetres in diameter and 15 to 18 centimetres long. To attach the wire, heat the end of the rod to redness, push one end of the wire into the soft glass for a short distance, and allow it to harden. The platinum wire should be about $\frac{1}{4}$ millimetre in diameter and 4 to 7 centimetres long. The "needle" has the wire straight, the "loop" has a loop or "oese" about 2 millimetres in diameter at the end.



FIG. 38.—Platinum Inoculating Needle and Loop, in glass handles ($\times \frac{1}{2}$).

Just before making inoculations with the needle or loop it is sterilized by heating it to a white heat in the flame, and passing the glass rod a few times to and fro in the flame; before use about ten seconds should be allowed to elapse, to allow the wire to cool sufficiently not to kill the germs picked up. The loop or the end of the needle is then touched to the bacterial growth or material to be inoculated; the wire is passed into a culture tube, the plug being temporarily removed and held by its outer end between two fingers, and the material adherent to the loop or needle is deposited in or on the surface of the culture medium, avoiding contact with the sides of the tube, after which the plug is replaced. Before inoculating them, it is well to heat the upper half of the culture tubes and flame the plugs. After inoculation, tubes should be labelled for identification. Inoculated tubes should be kept upright, which is conveniently accomplished by standing them in tin boxes or ordinary drinking-glasses with

a layer of cotton in the bottom.

In transplanting a growth from one tube to a fresh tube, the two are held upright side by side in the left hand, and their plugs removed and held by their outer ends between the sides of the disengaged fingers of the left hand. The platinum wire, held in the right hand, is sterilized, passed into the fertile tube, and a small portion of the growth taken on the end of the wire; the wire is then withdrawn, passed into the fresh tube, and this inoculated, after which the plugs are replaced.

In inoculating *liquid* media (bouillon, milk), the loop or needle is passed into the fluid and stirred around to dislodge the bacteria and mix them with the culture medium. Inoculations on the *surface* of solid media (agar slants, blood serum, potato) are made by drawing the loop or point of the needle, charged with bacterial material, over the surface. *Streak cultures* are those made by drawing the end of the charged needle over the surface of the medium in a straight line, so that the resulting bacterial growth develops in a line or streak. *Stab cultures* are made in the *depths* of solid media, usually gelatin, by plunging the needle, with its end charged with bacteria, for a few centimetres in a straight line into the medium and then withdrawing it along the same line.

Occasionally the culture tubes are inoculated otherwise than with the platinum wire, as by rubbing bits of tissue, held in sterile forceps, over the surface of solid media, or by dropping pieces of tissue or of ligatures, dressings, etc., whose sterility is in question, into liquid media. Swabs made of cotton twisted on the end of a wire, kept in a plugged test tube and so sterilized, are sometimes used for making inoculations, as in examining for diphtheria bacilli.

Growth of Cultures.—After inoculation, a variable time is required for the development of colonies of bacteria, usually from one to three days. Proper conditions of temperature, access of oxygen, etc., must be afforded to obtain the optimum growth.

As to temperature, cultures are ordinarily grown either at the room temperature, or in an incubating or culture oven at a temperature of 37°C ., or body heat. Most pathogenic germs grow better or more rapidly at 37°C . Gelatin cultures can be grown only at room temperature, below 25°C .; other media can be used at any temperature.

Culture ovens (Fig. 39) are specially constructed for the cultivation of bacteria. These ovens have jacketed walls, and are surrounded with a space filled with water. Heat is applied by a small gas flame beneath, provided with an automatic cut-off which stops the flow of gas should the flame be accidentally extinguished. By means of an adjustable regulator, or "thermostat," the flow of gas is so controlled that any desired temperature can be constantly maintained. A thermometer passing through the top of the apparatus registers the temperature.

Some bacteria (*aerobic*) grow only (obligate aerobic) or best (facultative) in the presence of oxygen; others (*anaerobic*) grow only (obligate anaerobic) or best (facultative) when free oxygen is excluded. As ordinarily grown cultures are aerobic; anaerobic cultures require special methods (see below).



FIG. 39.—Culture Oven for Bacteria. (Eimer & Amend.)

Isolation of Bacteria in Pure Culture.—When different kinds of bacteria are mixed or supposed to be mixed together in any material to be investigated, it is in general necessary to separate or isolate each kind in pure culture in order to investigate or determine the various kinds present. In some cases bacteria can be isolated by animal inoculation, or by the use

of special culture media or conditions which favor the growth of some varieties at the expense of others. The isolation of bacteria is usually, however, accomplished by "plate cultures," also by a less formal "streak method."

Plate Cultures.—For this method Petri plates (Fig. 40) are used. These are flat, shallow glass dishes which come in pairs, one being slightly larger than the other and serving as a cover for it. The cover when in place prevents the access of bacteria to the lower dish, which contains the culture medium. The dishes are sterilized by heating them in the hot-air oven two or three hours, and numbers of them should be kept on hand sterilized and ready for use; the oven itself is a convenient place to store them.



FIG. 40.—Petri Plate. (Eimer & Amend.)

Plate cultures are made with either agar or gelatin, agar having the advantage of being capable of use in the culture oven. Three sterile plates and three tubes of the medium are selected. The culture medium is then melted in the tubes; if gelatin, by standing the tubes in hot water; if agar, by means of the steam sterilizer, or by heating them carefully over a Bunsen flame and then placing them in a hot-water bath. The melted material is then cooled below 50° C. and inoculated while between that temperature and the solidification point.

The three tubes are inoculated as follows: Into one tube (the "original" tube) are placed two or three loopfuls of the material under investigation, which is thoroughly stirred up with the melted culture medium. The platinum loop being sterilized, two or three loopfuls of the material in the original tube are transferred to the second tube (the "first dilution"), and well mixed up in that. In like manner two or three loopfuls are transferred from the second tube to the third tube (the "second dilution"). The cotton plugs being removed, the upper portion of each of the tubes is well heated in the flame and the contents of the tubes are poured into the Petri dishes, the covers being lifted momentarily for the purpose and each tube being emptied into a separate plate. It should be remembered which is the original and which the first or second dilution, and the plates labelled accordingly. The culture medium is caused to form a level layer in the bottom of the Petri plate, allowed to harden, and the whole is set aside either in the oven or at room temperature to await development of the colonies of bacteria. The covers of the plates should be kept carefully in place and removed only very briefly in the necessary manipulations.

By this procedure it will usually be found after the bacteria develop that in one of the three plates the colonies (each of which is a pure culture originating from a single bacterium) will be sufficiently scattered and separated to enable them to be studied individually or transplanted to separate tubes, without mixture, and in this manner the varieties present can be isolated and identified.

The plate method is also useful for examination of the appearance of discrete colonies originating from a single bacterium, located both on the surface and in the depths of the medium.

Streak Method of Isolating Bacteria.—While this method is

more extemporaneous and less formal than the classical plate method, it is often an effective means of isolating bacterial species, is a simpler and less troublesome procedure, can be employed with blood serum as well as with agar, and is hence available for certain important bacteria that do not grow well on agar, and is easily adapted to anaerobic methods.

It is carried out thus: The platinum needle is charged with the material to be inoculated, and is then drawn a number of times in different places over the surface of the blood-serum or agar slant or other medium employed. Usually, at some point the bacteria will be so rubbed off from the platinum needle that single bacteria will be deposited here and there, from which isolated colonies will grow and can be studied or transplanted separately.

Fermentation Tube.—For investigating the power of bacteria to cause the fermentation of sugars, with the generation of carbon dioxide, the fermentation tube (Fig. 41) is the best means. This consists of an upright arm closed above and opening into an overflow bulb below. Glucose bouillon (or other sugar bouillon) is poured into the apparatus, sufficient to fill the long arm, with enough in the overflow bulb to prevent the fluid in the arm from escaping. A cotton plug is inserted into the mouth of the bulb, and the whole is sterilized in the usual manner. The tube is inoculated by depositing the bacteria with the platinum loop in the bouillon at the bottom of the bulb. If the bacterium is one that ferments sugar, gas will gradually collect in the upper part of the long arm; if it does not ferment sugar, no gas will appear.



FIG. 41.—Fermentation Tube. (Bausch & Lomb.)

The amount of gas produced can be easily measured. The amount of CO_2 in the gas formed can be ascertained by pouring 2-per-cent sodium-hydrate solution into the bulb till it is completely filled, closing the mouth with the thumb, well mixing the liquid and gas, returning the gas to the long arm, and then removing the thumb; the loss of volume suffered by the gas represents the amount of CO_2 that had been present and was absorbed by the alkali.

Fermentative processes can also be observed in stab cultures in glucose gelatin, in which gas bubbles develop under the action of fermentation-causing bacteria.

Anaerobic Cultures.—Various methods and forms of apparatus have been devised for removing free oxygen from contact with cultures, among which Buchner's pyrogalllic-acid method is simple and practicable. It is carried out thus: Large test tubes, about 2.5 by 25 centimetres in size, are employed, large enough to contain the ordinary culture tubes. Into the bottom of each of the large tubes is placed a small vial, or glass spool, or piece of glass rod, to support the bottom of the contained culture tube a few centimetres from the bottom of the outer tube. For every 100 cubic centimetres of air space in the large tube, there is then placed in the bottom of the tube 1 gram of pyrogalllic acid, and upon this 10 cubic centimetres of a .6-per-cent solution of potassium hydrate. As quickly as possible the inoculated culture tube is introduced into the large tube and the latter closed airtight with a rubber stopper. The pyrogalllic acid absorbs the contained oxygen, turning black in the process, and anaerobic bacteria are enabled to grow.

Indol Formation.—Some bacteria, as the colon bacillus, cholera spirillum, and other putrefactive bacteria, possess the power of forming indol as a product of their growth. To test the power of indol-formation Dunham's peptone solution is usually employed, consisting of pure, dry peptone 1 per cent, sodium chloride .5 per cent, water 100 parts; neutralization is not necessary; about 7 cubic centimetres is placed in each tube and sterilized. Carbohydrates (which may be tested for by the copper test) and other impurities should be absent. Indol should be shown to be absent from the culture medium by control tests.

Three or four tubes are inoculated and after one to four days tested for indol. This is done by adding 10 drops of strong sulphuric acid to a tube; if a rose color develops both indol and a reducing substance (nitrous acid) have been produced. If no rose color appears after a few minutes, add 1 cubic centimetre of a .01-per-cent solution of sodium nitrite; if a rose color now appears, indol was produced by the bacteria, but no nitrites. If no rose color forms indol is absent.

Cultural Characters of Bacteria.—One of the main objects of isolating bacteria and growing them on various media is to determine their cultural characteristics, as an aid to identifying the species under investigation, or for other purposes. These characteristics relate both to the gross appearance or morphology of

the cultures and to the chemical products or changes arising from the growth of the germs.

With reference to the appearance of the growth on solid media, the color, form, viscosity, degree of moisture or dryness, size or extent of the colony, and fluorescence are among the points to be noted, both as to superficial or buried colonies on gelatin or agar plates, stab cultures in gelatin or agar, or streak cultures on agar slants, blood serum, or potato. In liquid cultures (bouillon) the formation of general cloudiness, flocculi, surface scum, or sediment, are among the points to be observed. The rate of development at different temperatures, the most favorable temperature for growth, the aerobic or anaerobic qualities, are also specific characteristics.

Among the chemical products and effects to be noted are the odor, the power of liquefying gelatin, the production of acids (as shown in litmus-milk or bouillon cultures, etc.), the power of coagulating milk, the power of fermenting sugars and generating gas, the production of indol.

Determination of Number of Bacteria in Liquids.—To ascertain the number of bacteria in definite volumes of water, milk, or other liquids, a small amount of the fluid is accurately measured off, diluted if necessary to a sufficient degree (to be ascertained by trial) to bring the bacteria within manageable numbers, and a measured amount of the dilution mixed with the melted contents of an agar or gelatin tube, which is then poured into a Petri plate, allowed to harden, and grown in the usual manner. The dilution must be made with sterile water, and the manipulations carried out aseptically. The number of colonies which develop on the Petri plate represents the number of bacteria in the portion of the original fluid that was introduced into the plate.

B. MICROSCOPICAL METHODS.

The morphology, size, motility, staining properties, presence of flagella or capsules, and power of spore formation of bacteria are determined by direct microscopical examination with the oil-immersion objective. Bacteria may be examined microscopically either in the fresh or stained condition, and either in sections of the solid tissues, in pus, sputum, or other body fluids, or from growths on culture media.

Sections of tissues intended to show bacteria are prepared in the usual manner, but should be thin. The staining methods are the same as those given below. Pus and other fluids containing bacteria are examined by the cover-glass methods already referred to, being prepared, fixed, and stained in a similar manner as are bacteria taken from growths on culture media, now to be described.

Bacteria from growths on culture media are examined either fresh or stained.

To examine them **fresh**, if the culture is liquid (bouillon or liquefied gelatin) a drop or two is taken with the platinum loop, placed on a slide, and covered with a cover-glass. If the culture is on a solid medium, two or three loopfuls of sterile distilled water are placed on the slide; a minute portion of the growth is taken on the end of the platinum wire, and mixed evenly with the water, over which a cover-glass is then placed. The specimen is examined with the oil-immersion objective with dim sub-stage illumination. Or if preferred a "hanging-drop" may be prepared as described in connection with the Widal test (page 62), on the lower surface of the cover-glass placed over the concavity of a "hollow-ground slide"; the long working distance is apt to prevent the use of an objective as high as the 2-millimetre oil-immersion with the hanging-drop. Examination of bacteria in the living state yields information especially as to their motility, besides exhibiting their morphology.

Staining Bacteria.—The first step in the process of staining bacteria is the *preparation of cover-glass spreads*. With liquid cultures two or three loopfuls of the fluid are deposited on a cover-glass, spread around with the loop in a thin layer, and allowed to dry. If the growth is on a solid medium, a like amount of sterile distilled water is placed on the cover-glass with the loop; a minute portion of the growth is transferred to this water on the point of the platinum needle, and with the loop the whole is well mixed and spread out, by circular movements of the loop, in a thin layer, which is allowed to dry. If it is desired to hasten the drying, the glass may be held in the fingers high over a flame, but should not be heated to a point hotter than the fingers can stand. It is important that the glass be clean and free from fat, otherwise the fluid will not spread well. If preferred the bacteria can be spread on a slide and the cover-

glass dispensed with entirely. Spreads once prepared can be kept indefinitely before further treatment.

The spreads being thus prepared and dried, the next step is *fixation*. In bacteriological work this is done by flaming the specimens, that is, by grasping them near the edge with forceps and passing them three times through the Bunsen flame at moderate speed at brief intervals.

They are now ready for *staining*, for which a number of stains and processes may be used. To stain, a small amount of the staining solution is placed in a watch-glass, and the cover-glass immersed in it or floated on it with the film in contact with the stain; or the cover-glass, held in the grasp of self-retaining forceps, is covered with as much of the stain as it will hold. If heat is necessary, the watch-crystal or cover-glass thus arranged may be heated over a flame.

After being acted on by the stain for a sufficient time, the fluid is washed off with water. For temporary examination the wet cover-glass is placed film down on a slide, its upper surface dried with filter-paper, and examined with the oil-immersion lens. For permanent mounts the cover-glass is dried and mounted with balsam.

Impression preparations are made by pressing a clean cover-glass lightly on the surface of a colony, and lifting it off again by a direct upward movement. The bacteria adhere in the same relations in which they grew, and may be so studied.

The principal stains employed for bacteriological purposes are as follows:

Methylene blue: This is chiefly employed in the form of *Loeffler's solution*, as follows:

Saturated alcoholic solution of methylene blue.....	30
1:10,000 solution of potassium hydrate.....	100

This solution is applied for three to fifteen minutes until the specimen is sufficiently stained.

Watery solutions of methylene blue (1 or 2 per cent) may be used. Gabbet's solution with 25-per-cent sulphuric acid has been mentioned in connection with the examination of sputum for the tubercle bacillus.

Fuchsin: This is usually employed in solution with phenol (*carbol-fuchsin*) as follows:

Saturated alcoholic solution of fuchsin.....	10
5 per cent solution of phenol in water.....	100

It is well to prepare the solution fresh from stock solutions every few weeks. Carbol-fuchsin is employed full strength, with heat, in staining the tubercle bacillus (page 129); but for ordinary bacteria it should be diluted with several times its volume of water to a light transparent red, and applied for from one to five minutes.

Gentian violet: A powerful staining solution that will keep indefinitely may be prepared as follows:

Gentian violet.....	1
Formalin (= .4 formaldehyde).....	1
Water.....	100

This stains deeply in from ten to sixty seconds.

Gentian violet and other anilin dyes are often used in solution in anilin-water. Anilin-water is prepared by well mixing 5 parts of anilin oil with 95 parts of water and filtering through moistened filter-paper. The staining fluid is prepared by mixing 10 to 15 parts of a saturated alcoholic solution of gentian violet (or fuchsin, etc.) with enough anilin-water to make 100 parts. The solution keeps only a few days.

Stirling's gentian-violet solution, which is much used and is said to keep well, is as follows:

Gentian violet.....	5 grams
Alcohol.....	10 c.c.
Anilin oil.....	2 "
Water.....	188 "

Most bacteria stain well with any of these three stains, both in sections and in cover-glass preparations. Methylene blue is an excellent nuclear stain; fuchsin and gentian violet are diffuse cellular stains and do not show tissues well.

Gram's method of staining is employed for the differentiation of certain bacteria, also to show the capsule of the micrococcus lanceolatus and other germs. It is carried out thus:

1. Stain with gentian violet in the manner just indicated.
2. Rinse with water.
3. Immerse for one-half to two minutes in a solution of 1 part of iodine and 2 parts of potassium iodide in 300 parts of water.

4. Immerse in 95-per-cent alcohol a few minutes so long as color is discharged.

5. Wash with water and mount.

When thus treated some bacteria (as the pyogenic staphylococci, streptococcus pyogenes, micrococcus lanceolatus, diphtheria bacillus, tubercle bacillus, anthrax bacillus, tetanus bacillus) retain the stain, while other bacteria become decolorized (as gonococci, typhoid bacillus, colon bacillus, influenza bacillus, cholera spirillum).

Staining Flagella.—Several methods of staining the flagella of motile bacteria have been introduced, among which may be mentioned that of Loeffler. The cover-glass films must be very thin and the bacteria well separated. This may be insured by a dilution method, thus: a drop of water is placed on each of three or four well-cleaned cover-glasses; a portion of the culture (best on agar, eighteen or twenty hours old) is mixed with the water on one cover-glass; a portion of this is transferred with the needle to a second cover and mixed with the water; a like transfer is made from the second to the third, etc. On one of the cover-glasses the bacteria should be found properly spread. After drying and fixing, the film is treated with the following mordant about a minute, being warmed over a flame, but not boiled:

Twenty-per-cent solution of tannic acid in water.....	10
Saturated aqueous solution of ferrous sulphate (fresh).....	5
Saturated watery or alcoholic solution of fuchsin	1

After mordanting, wash with water and stain with a saturated solution of fuchsin in anilin-water.

Staining of Spores.—Spores do not stain well by ordinary methods, yet they usually show unstained. If it is desired to stain them, various methods may be used, such as Abbott's, which is as follows: The fixed cover-glass preparation is stained with Loeffler's alkaline methylene-blue solution for a minute with the aid of heat, the fluid being boiled at brief intervals. After washing in water it is decolorized in a mixture of 2 parts of nitric acid with 98 parts of 80-per-cent alcohol till the visible blue color is discharged. After washing, it is stained three to five seconds in a mixture of 10 parts of saturated alcoholic solution of eosin with 90 parts of water. It is then washed and mounted. The spores are stained blue and the bacterial cell bodies red.

C. ANIMAL INOCULATION.

The objects of animal inoculation are to determine the pathogenic action of bacteria, produce antitoxins, increase the virulence of bacteria, isolate bacteria in pure culture, etc. For clinical purposes animal inoculation is occasionally practised for diagnostic purposes, especially in connection with suspected rabies and tuberculosis.

Rabbits, guinea-pigs, and mice are most often employed. The inoculations may be made with bacteria from cultures on solid media, with bacteria suspended in liquid, with pathological exudates like sputum or pus, or with bits of suspected tissues. The inoculations are made into the subcutaneous tissues, the peritoneum, a vein, under the dura mater, or elsewhere. While being inoculated the animal may be held by an assistant, whose hands if necessary are protected by a towel folded thick, or it may be held in one of the "animal holders" devised for the purpose.

Subcutaneous Inoculations.—With rabbits and guinea-pigs these are usually made over the abdomen, with mice at the root of the tail. The hair is clipped short at the site of inoculation; attempts at cleaning and disinfection are not usually made. Liquid bouillon cultures or other fluids may be injected with a sterilized hypodermic syringe. If solid material is to be introduced a small snip is made in the skin and subcutaneous tissues with sterilized scissors; sputum, pus, or bacteria from solid culture media are then introduced with the platinum loop, or bits of tissue with sterile forceps. The material is deposited in the tissues as far from the opening as possible, avoiding touching the edges of the wound. No further care of the wound is necessary, except perhaps a little collodion.

Intraperitoneal Inoculation.—With liquids, the skin is nicked in the median line, and through this opening the injection is made with a hypodermic syringe into the peritoneal cavity. Solid material is introduced through a minute laparotomy wound made aseptically under ether anæsthesia.

Intravenous Inoculation.—This is performed by means of a hypodermic needle introduced into the venous branch near the posterior margin of the rabbit's ear, the needle being inserted near the apex and from the dorsal surface of the ear.

Intracranial inoculation is practised with emulsions of the brain and spinal cord in order to determine if suspected animals are affected with rabies (see page 282).

After inoculating animals by any method, the symptoms and conditions that develop are carefully observed, and at death or after killing the animal the requisite bacteriological and histological examinations are made.

D. CHARACTERS OF PATHOGENIC BACTERIA.

The chief application of bacteriological methods for diagnostic purposes is the determination of the presence of bacteria and the identification of the species of such as may be found in morbid conditions. Species of bacteria are clinically identified chiefly by means of the characteristics revealed by microscopical examination and cultures. Consideration of the source from which they were derived is also important. Microscopical ex-

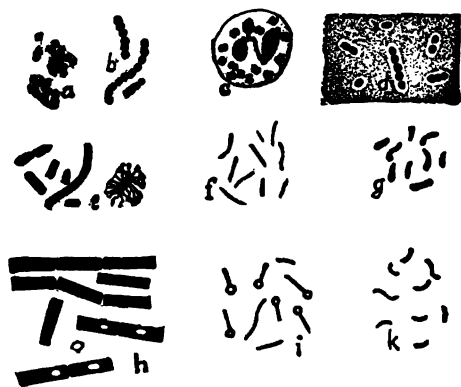


FIG. 42.—Common Pathogenic Bacteria ($\times 1,000$). a, Staphylococci; b, streptococci; c, gonococci, in leucocyte; d, micrococcus lanceolatus (pneumococcus); e, typhoid bacilli, one showing flagella; f, tubercle bacilli; g, diphtheria bacilli; h, anthrax bacilli, some with spores; i, tetanus bacilli; k, cholera bacilli.

amination alone is often sufficient to identify the germ, while resort to animal inoculation is occasionally required.

The commonest pathogenic bacteria, with their distinguishing morphological, cultural, and pathogenic characteristics, are briefly as follows:

Staphylococcus pyogenes aureus: A coccus, about $.8 \mu$ in diameter, occurring singly, in pairs, or in irregular clusters. Stains with ordinary stains, and does not decolorize by Gram's method.

It grows readily at from 20° to 37° C. on agar, potato, and blood serum as shining, moist, slightly elevated colonies, at first pale in color, but in the course of two or three days becoming yellow or orange. The yellow color develops best in colonies grown in the dark at room temperature. Individual colonies are rounded, a

few millimetres in diameter; streak cultures are a few millimetres wide.

It liquefies gelatin. In stab cultures in gelatin liquefaction begins in twenty-four to forty-eight hours; first at the top of the gelatin, and then extending laterally and downward, assuming a conical form with apex below. A yellow sediment collects at the bottom, and in four or five days the gelatin is entirely liquefied.

In bouillon a diffuse cloud develops, with a yellow sediment later. Litmus-milk is turned red and coagulated. It does not ferment sugar or produce gas.

This bacterium very frequently causes and is found in abscesses, suppurative, inflammatory, and infectious conditions in various situations, septicæmia, and pyæmia. Injection into the circulation of animals is followed by fatal pyæmia and metastatic abscesses, especially in the voluntary muscles and kidneys; subcutaneous or intraperitoneal inoculations may produce local lesions or not, according to the virulence of the culture and the extent of disturbance of the circulation or injury of the tissues at the site of inoculation.

In practice this germ is identified mainly by its cultural characters, after isolation in pure culture.

Staphylococcus pyogenes albus: This is similar in most respects to the preceding organism, but its colonies and pigment are white and it is less virulent and pathogenic. It is especially abundant in the epidermis.

Streptococcus pyogenes: This is a rounded or sometimes flattened micrococcus that grows in straight or wavy chains, varying in length from short chains of three or four bacteria up to those that are very long. Their size is variable, ranging from .4 to 1μ ; in the same chain different elements may vary in size. Sometimes a transverse line of division is present. It stains with the ordinary stains, and is not decolorized by Gram's method.

It does not grow well on ordinary media, and is apt to lose its vitality quickly. It grows best on blood serum and glycerin-agar at 37°C ., and better on media prepared directly from meat than on those made with meat-extract. Its growth on blood serum, agar, and gelatin consists of minute grayish-white, shining, translucent, round colonies. It does not liquefy gelatin, and in stab

cultures grows in the form of minute granules or colonies along the line of inoculation, with little surface growth. In bouillon it may cause general cloudiness or grow in clumps, and sometimes reddens or coagulates litmus-milk.

The germ causes erysipelas, abscesses, suppurative, inflammatory, or infectious lesions, catarrhs of the pharynx and respiratory tract, and septicæmia, and may be found in the tissues, pus, exudates, and fluids in these conditions. It varies in virulence, and the effects of animal inoculation are inconstant.

In clinical work the streptococcus is identified most often by its characteristic appearance in stained specimens of exudates; occasionally its presence is revealed by blood-serum cultures.

Gonococcus: This is a diplococcus, averaging about .7 by 1.25 μ in size, consisting of two flattened hemispherical portions separated by a narrow interval. In gonorrhœal pus these bacteria are most characteristically found within the bodies of the leucocytes, often crowded together in large numbers; only a certain proportion of the leucocytes contain the germs, most of the pus cells being free from them. The gonococci may also be free in the pus plasma. They stain well with ordinary stains (especially Loeffler's methylene blue), but decolorize by Gram's method.

Gonococci do not grow on ordinary culture media, but require special media containing blood serum (especially human blood serum). Ordinary animal inoculations yield negative results.

Gonococci occur in gonorrhœal pus from the genitals and eye, and are occasionally associated with articular, endocardial, and other lesions. For clinical purposes this bacterium is identified in stained specimens of pus by its form, staining properties (decolorization by Gram's method), and its characteristic localization within leucocytes.

Diplococcus intracellularis meningitidis: This bacterium is similar in morphology and staining reactions to the gonococcus. It decolorizes by Gram's method. It is associated with infectious cerebro-spinal meningitis, and is found in the cerebro-spinal fluid and meningeal exudates in that disease, being present both inside and without the leucocytes. It has also been found in the nasal secretions. On blood serum it forms small, round, colorless colonies, which may become confluent. On other media it grows feebly and quickly dies out.

Micrococcus tetragenus: This is a micrococcus, occurring most

characteristically in fours, which are enveloped in a transparent gelatinous matrix. It is frequently observable in sputum, especially in tuberculous cases.

Micrococcus lanceolatus (commonly called the pneumococcus, or diplococcus of pneumonia): This is a micrococcus of ovoid, "lancet-shaped" (pointed at one end), or rounded form, surrounded by a capsule. It occurs singly, in pairs, or in short chains, but it appears most frequently and characteristically in pairs, separated by an interval and with the broad ends of the ovoids toward each other. The capsule shows best in preparations from the blood or sputum, but is not always evident, and in cultures is not ordinarily seen.

It stains by the ordinary methods, and also by Gram's method, which shows the capsule nicely. After the application of Gabbet's method for the tubercle bacillus the pneumococcus may appear conspicuously.

The micrococcus lanceolatus grows very feebly on ordinary culture media, and rapidly dies out, so that to keep it alive it must be transplanted every two or three days. It grows best on blood serum at 37° C. in the form of very minute, colorless, transparent colonies.

This micro-organism is frequently present in the mouth of healthy persons, and its appearance in the sputum is not necessarily of pathological import. In croupous pneumonia it is present in the sputum in large numbers, and also occurs at times in suppurative and inflammatory conditions in other situations, as in pleurisy, endocarditis, meningitis, otitis media, etc.

Subcutaneous inoculation of rabbits or mice with tissue, sputum, or other exudate containing virulent lanceolate micrococci is followed by a septicæmia fatal in twenty-four or thirty-six hours, and the organism can be recovered from the blood. Inoculation with cultures produces septicæmia, local inflammatory reaction, or no result at all.

Clinically, this germ is easily recognized microscopically in stained specimens of exudates containing it; occasionally cultures on blood serum or animal inoculation may be required to reveal its presence.

Tubercle bacillus: This is a slender bacillus, straight or slightly bent, about .2 μ in diameter and 2 to 4 μ long. In stained specimens its continuity usually appears somewhat broken, inter-

rupted, or uneven, giving it a beaded appearance. On blood serum at 37° C. its colonies develop very slowly, appearing after two or three weeks and forming a dry, cream-colored, membranous or scaly growth; it also grows very slowly on glycerin agar and in glycerin bouillon, but not on other media.

Clinically the tubercle bacillus is in the great majority of cases detected by staining methods; occasionally by resort to animal inoculation.

This bacillus resists staining by the ordinary methods; but when once stained it is equally resistant to decolorizing agents. On this depends its specific method of demonstration. The suspected material is first stained with boiling carbol-fuchsin, by which tubercle bacilli are deeply stained; it is then treated with nitric or sulphuric acid, diluted three or four times, for thirty to sixty seconds. By this treatment everything is decolorized except the tubercle bacilli, which remain red; by treatment with methylene blue other bacteria, nuclei, etc., are then stained blue for contrast. By Gabbet's method the decolorizing and counter-staining are combined in one procedure. The details of these methods of staining for the tubercle bacillus are given in connection with the sputum (page 129).

The smegma bacillus and the leprosy bacillus possess staining properties similar to those of the tubercle bacillus, and may therefore be confounded with the latter. The leprosy bacillus is the most difficult to distinguish from the tubercle bacillus, but is very rarely encountered. In specimens from the genito-urinary organs, male or female, it is always necessary to distinguish between tubercle bacilli and smegma bacilli. This may be done by immersing the cover-glass specimen, after staining with fuchsin and decolorizing with acid, in 95-per-cent alcohol for about thirty seconds; this treatment decolorizes smegma bacilli but leaves tubercle bacilli stained red as before.

In clinical work occasion arises for examining for tubercle bacilli in sputum, urine, fæces, pus, cerebro-spinal fluid, etc. When present in sputum they are usually easily demonstrated, but in urine, pus, and other exudates they are apt to be in such small numbers that their detection is often very difficult. In examining urine, genito-urinary discharges, or fæces for tubercle bacilli it is necessary to treat the specimen with alcohol in order to differentiate and exclude smegma bacilli.

Suspected material in which the bacilli are difficult to demonstrate microscopically, from their scarcity or other cause, may be tested for the presence of tubercle bacilli with much certainty by animal inoculation. Guinea-pigs or rabbits (preferably the former) may be used, and the material to be tested inoculated subcutaneously. If the bacilli are present, tuberculosis will develop and in a few weeks be manifested by emaciation, enlargement of the nearest lymphatic glands (in which the bacilli should be demonstrable), dyspnoea, etc., with death a little later.

Diphtheria bacillus: This bacillus occurs in the membranous exudate of diphtheria, and examination for it in pharyngeal pseudo-membranes is an important diagnostic procedure. The method is as follows:

The culture medium employed is Loeffler's blood serum (page 259). The tubes are inoculated by means of a sterilized swab prepared by twisting a little cotton on the end of a wire; the swab is inserted into a test tube which is closed with a cotton plug through or alongside which the end of the wire projects; thus enclosed in a plugged test tube the swab is sterilized with steam. To inoculate the tube, the swab is touched or rubbed on the pseudo-membrane in the pharynx, and the adherent portion of the exudate is smeared over the surface of the serum in a culture tube. The swab is immediately burned or returned to its tube to prevent dissemination of the germs.

The tube is set away at 37° C. for cultivation. The diphtheria bacillus grows so rapidly on this medium that, if present, in from eighteen to twenty-four hours it presents quite characteristic colonies. These are large, elevated, rounded and discrete or confluent and irregular, grayish or creamy white, moist (becoming drier in two or three days), and with a centre denser than the periphery.

The diphtheria bacilli in these colonies when stained with Loeffler's methylene blue or by Gram's method present a characteristic morphology by which they may be identified. They exhibit considerable variation and irregularity in form. They are usually slightly curved, but may be straight, or bent and twisted. They may be clubbed or enlarged at one or both ends, fusiform, or irregular in shape, only exceptionally uniform. They stain heterogeneously and irregularly; each bacillus presents areas deeply stained, separated by lightly stained intervals, giving a

beaded or segmented appearance, or the appearance of two or three dark points at the ends of or scattered along a lightly stained rod. The size varies, but averages .6 or .7 by 2 or 3 μ .

Bacilli exhibiting this characteristic irregular morphology, in from eighteen to twenty-four-hour colonies on Loeffler's blood serum inoculated from the throat, can be quite safely regarded as diphtheria bacilli. Innocuous bacteria at times, however, appear from the mouth or throat simulating diphtheria bacilli in appearance and causing doubt and confusion in diagnosis. A crucial test for the virulence of these bacilli can be made by subcutaneous inoculation of guinea-pigs, which are killed by virulent diphtheria bacilli in from one to five days through the production of a local inflammation and general toxæmia.

By Neisser's method of staining it is said that true diphtheria bacilli can with considerable certainty be distinguished as such. The cultures, on Loeffler's blood serum, are grown at from 34° to 36° C. for not over twenty-four hours. The cover-glass preparations are stained as follows:

a. Stain one to three seconds in:

Five-per-cent alcoholic solution of methylene blue (Gruebler's)...	2
Five-per-cent aqueous solution of acetic acid.....	100

b. Wash thoroughly in water.

c. Stain three to five seconds in a .2-per-cent solution of Bismarck brown in boiling distilled water, filtered and cooled.

d. Wash and mount.

Thus treated, diphtheria bacilli appear as faint brown rods containing one, two, or three oval dark-blue granules.

The diphtheria bacillus grows less vigorously on other media than Loeffler's blood serum, and grown on these media it has different and less distinctive morphological and staining characteristics.

Typhoid bacillus: A bacillus with rounded ends, .6 or .7 μ in diameter, 1 to 3 μ long, or sometimes growing in long filaments; sometimes short oval in form. It is actively motile in young cultures, and possesses numerous flagella demonstrable by special staining methods. It does not form spores. It stains by the ordinary methods, and decolorizes by Gram's method. It usually (but not invariably) agglutinates and loses its motility under the action of blood serum from a typhoid-fever patient.

It grows well on media at 20° to 37° C., but rather more slowly than the colon bacillus. Surface colonies on gelatin are small, whitish, with irregular margins, a corrugated surface, and a

dense central point. Stab cultures in gelatin grow in a narrow line along the line of inoculation, spreading a little on the surface. It does not liquefy gelatin, or ferment any sugar with gas-production, or ordinarily produce indol. Streak cultures on agar slants are a few millimetres wide, whitish, shining, moist. It does not redden or coagulate litmus-milk. On potato it forms a usually "invisible" growth. It clouds bouillon, with some deposition of sediment.

Animal inoculation is almost always negative, though injection of excessive amounts may produce toxic results.

In cases of typhoid fever, typhoid bacilli are discharged with the faeces and often in the urine. Special methods of detecting the presence of these bacilli in the faeces with sufficient quickness and certainty to be of diagnostic utility have been presented, as those of Elsner and Piorkowski, but have not come into general use. The production of agglutinins by the typhoid bacillus and the Widal method of their demonstration have been considered in connection with the blood. After death, the typhoid bacillus is obtainable from the spleen and other organs; and in atypical typhoid infections, as abscesses, osteomyelitis, meningitis, etc., it is found in the affected areas or exudates.

The certain recognition of typhoid bacilli from the body or water supplies is often a difficult matter. It is especially apt to be associated and confounded with the colon bacillus. To identify it, it is necessary to separate it in pure culture and then carefully test and consider all its morphological, vital, and cultural characteristics. The chief crucial points of distinction from the colon bacillus are the action on sugar and formation of gas, and the action on litmus-milk. The reverse of the Widal method may be employed to test the bacilli under examination, by treatment with typhoid blood serum known to cause agglutination of typhoid bacilli; but even this method (especially a negative result) is not always reliable.

Colon bacillus (*bacillus coli communis*): This is a constant and normal inhabitant of the intestine and in other infections often makes its way into the organs. It is ordinarily innocuous, but may be the cause of suppurative or inflammatory conditions in the abdominal, pelvic, genital, and perineal regions.

In appearance and size it resembles the typhoid bacillus, being a rod with rounded ends, sometimes so short as to be

almost spherical, sometimes growing in long filaments. Flagella are present, fewer than in the case of typhoid bacilli; and the colon bacillus is capable of motion, though often its motility is sluggish and doubtful. It stains with the usual dyes, and decolorizes by Gram's method.

It grows readily at 20° to 37° C. Separate colonies on the surface of gelatin are small, irregularly rounded, with irregular margins, dryish, whitish, with a dense or nucleus-like centre. It does not liquefy gelatin. Gelatin stab cultures have a "nail-like" form, growing along the line of inoculation with a slight expansion on the surface; in time, lateral branches may grow in the depths of the gelatin. Its growth in bouillon and on agar slants also resembles the growth of typhoid bacilli.

On potato the colon bacillus produces a voluminous gray or brownish growth. Litmus-milk at 37° is turned red in about twenty-four hours, and in a little longer time is usually coagulated. Sugars, especially glucose, are vigorously fermented, with voluminous production of gas; this is a very characteristic feature. It produces indol markedly in peptone solution in a day or two. Animal inoculation is very variable.

The identification of the colon bacillus is based on its various cultural characters.

Bacillus proteus vulgaris: A common putrefactive bacterium present in the intestine and sometimes associated with other germs in morbid conditions; may be pathogenic for animals. It is a bacillus of medium size, very variable in length, from short coccus-like forms to long filaments; motile. Decolorizes by Gram's method. Rapidly grows in and liquefies gelatin. In 5-per-cent (instead of the usual 10-per-cent) gelatin motile prolongations from the margin of the colonies grow out, and may become separated from the parent colony. Reddens and coagulates litmus-milk. Cultures generally have a putrefactive odor.

Cholera spirillum (comma bacillus): Occurs in large numbers in the intestinal contents in Asiatic cholera. A curved bacillus about .4 μ thick and .8 to 2 μ long; sometimes joined end to end in wavy forms. Motile, with a single flagellum at one end. Decolorizes by Gram's method. Grows rapidly on gelatin plates, with liquefaction. Gelatin stab cultures grow along the whole lines, with slow liquefaction at the top. Reddens and coagulates litmus-milk. Produces indol and nitrites.

Bacillus pyocyaneus: This occurs in the green pus sometimes found in abscesses, in diarrhoeal discharges, and elsewhere, and has variable pathogenic powers. It is a small bacillus with rounded ends, motile, not spore-forming. Decolorizes by Gram's method. Grows well on culture media, and usually imparts to the material in or on which it is growing a bright green color, gradually becoming darker. Liquefies gelatin, reddens and coagulates litmus milk, produces indol.

Influenza bacillus: Found in the respiratory exudate in influenza. A minute slender bacillus, about .2 by $.5\mu$ in size, non-motile, not spore-forming, sometimes growing end to end. Often situated within leucocytes. Stains with aqueous solutions of the ordinary anilin dyes, well with Loeffler's methylene blue, best with diluted carbol-fuchsin; may stain most deeply at the ends. Decolorizes by Gram's method. Grows on culture media only when blood (not merely blood serum) has been added. Blood obtained aseptically from a prick of the ear or finger may with a loop be spread over the surface of an agar slant, and this then be inoculated by rubbing it with a bit of mucus (rinsed in sterile water) from the sputum. The colonies grow at 37° as minute shining, colorless points.

Anthrax bacillus: This bacterium is rarely met with as a clinical infection, but is much used in the laboratory. It is a large bacillus, with square or concave ends, growing end to end in long filaments; it is 1 or 1.25μ thick and 3 to 20μ long, non-motile, and forms spores. It stains by the ordinary dyes, and is not decolorized by Gram's method.

Surface colonies on agar are formed of a dry matted layer of interlacing filaments, with irregular margin. It liquefies gelatin slowly. Inoculation of mice, rabbits, or guinea-pigs causes a fatal septicæmia.

Plague bacillus: Found in the blood, abscesses, and other lesions in bubonic plague. A short oval bacillus, 2μ long, sometimes growing in chains; non-motile, not spore-forming, sometimes encapsulated. Stains more deeply at the ends than in the centre; decolorizes by Gram's method. Does not liquefy gelatin, or ferment sugar, or produce indol. Coagulates milk.

Tetanus bacillus: A slender bacillus of variable length, sometimes growing in long filaments; motile. Forms spores, which are broader than the bacillus and situated at one end, giving it

a drumstick appearance. Stains with the ordinary dyes, and does not decolorize by Gram's method.

It grows slowly on culture media, and only under strict anaerobic conditions. In stab cultures in gelatin (the tubes are best filled about two-thirds full of the medium) delicate filaments grow laterally from the line of inoculation and the gelatin is slowly liquefied. It produces gas in media containing glucose. Subcutaneous inoculation of animals promptly produces fatal tetanus.

Bacillus aerogenes capsulatus (gas bacillus): This has been found in septic lesions, the female genitals, and in dead bodies, associated with emphysematous conditions in the affected tissues, the blood-vessels, and the viscera. It is a large bacillus, occurring singly, in pairs, clumps, or short chains; ends round, or square when joined; frequently shows a capsule. Stains by Gram's method. Grows only anaerobically; produces gas markedly in glucose-containing media; slowly and partially softens or liquefies gelatin; coagulates milk.

Rabies: The micro-organism that causes this disease has not been found. Animal inoculation affords a very useful and sure means of diagnosis in individuals or animals dead with rabies.

A small piece of the brain or spinal cord from the suspected case (preferably a piece from the floor of the fourth ventricle) is with a glass rod rubbed to a paste in a conical upright glass and well mixed with about ten times as much sterile bouillon or .6-per-cent sodium-chloride solution. The whole is done with strict asepsis and sterilized materials. After settling, the upper fluid is employed for inoculation.

The inoculations are made in rabbits or guinea-pigs beneath the dura mater. The skin and underlying tissues on the forehead of the animal are incised to the bone, the wound is held open, and with a small trephine 5 or 6 millimetres in diameter applied behind the orbit to one side of the median line, the dura mater is exposed. A syringe is used with a needle having its point bent at right angles; the dura mater can be pierced with this and then drawn outward so as not to injure the brain. With this syringe the emulsion of the suspected cord is injected under the dura. The wound is sutured. The whole operation is conducted aseptically.

If the case tested was one of rabies, the inoculated animal should show symptoms of the disease in six or seven days and die in twelve or fourteen days.

XIII. AUTOPSIES.

In autopsy work a cool, well-ventilated, well-lighted room is needed, with abundant water supply, a sink, and facilities for cleaning. The autopsy table should be about 200 by 90 by 75 centimetres in size, with raised edges and drainage from its centre. Scales for weighing the organs should be available.

A full outfit of instruments for autopsy work would include scalpels, a cartilage knife, scissors, forceps, a director, probes, chisels, a hammer, needles, saw, long knives for opening organs, a long, broad brain knife, a costotome for cutting the ribs, an enterotome, catheters, strong cord, sponges, etc.

A post-mortem examination begins with certain general observations. The report of the examination should include the name, date, the time elapsed since death occurred.

General inspection of the body reveals information as to race, sex, apparent age, size, general development, state of nutrition, color, post-mortem hypostasis of blood, rigor mortis, any abnormalities, deformities, traumatisms, distention of abdomen, the condition of the peripheral arteries, etc.

Examination of Thoracic and Abdominal Viscera.—To open the thorax and abdomen, make a median longitudinal incision through the skin from the top of the sternum to the symphysis pubis. If the entire trachea and oesophagus are to be explored, the incision is extended upward to the chin; but this is not often practicable. Over the abdomen the incision is carried through the muscles and peritoneum, exposing the abdominal cavity. Over the thorax, the flap of skin with all the underlying tissues and muscles is dissected up on each side, exposing the costal cartilages and ribs for a distance of eight or ten centimetres from the middle line. With the costotome or cartilage knife the ribs or costal cartilages are cut through on each side, near the costal junctions, taking care not to injure the underlying organs. The bone flap formed by the sternum and attached cartilages is freed from its attachments to the diaphragm below with knife or scissors, from its lateral muscular and ligamentous attachments, and from its attachments to the viscera beneath. As the dissection

proceeds the flap is lifted upward, swinging on the manubrium as a hinge; when entirely freed on both sides, the flap is bent upward out of the way, or removed entirely.

The contents of the thorax and abdomen are by these procedures freely exposed, and the viscera may be examined as a whole with reference to their position and relations. The thoracic and abdominal viscera are then removed and examined in detail.

Thorax.—The *pleura* on each side may be examined by inspection and by sweeping the hand around the cavities. The character of fluid present may be noted; and after removing it with a pipette or sponge its amount may be measured. The presence, position, and character of adhesions or other morbid conditions are also determined.

The *pericardium* may then be incised and its condition observed.

In cases of obscure sudden death the pulmonary arteries are best opened *in situ* to examine for emboli. Otherwise it is convenient to remove the left lung first, by breaking up or cutting adhesions and cutting the bronchi and vessels at the root of the lung. Extensive and firm adhesions may render removal of the lung very difficult.

The removal of the left lung permits the heart and entire thoracic aorta to be examined *in situ*, and greatly facilitates the determination of the existence and relations of any aneurisms. The heart is removed by severing the *venæ cavæ*, pulmonary vessels, and branches of the aorta, care being taken not to injure the auricles. As much of the aorta as is desirable may be removed with the heart. The portion of the aorta not removed may be subsequently slit open *in situ* and its interior examined.

The *heart* may be opened for examination after removal (or if desired *in situ*). The auricles and auricular appendages are first widely opened with scissors, one point being introduced through the cut venous openings. The auricular endocardium and valves are thus exposed. The right ventricle and pulmonary artery are then slit open by the enterotome or long scissors, one point being introduced through the artery into the ventricle; finally the right heart is still more widely opened by passing a blade of the scissors through the tricuspid opening and dividing the entire auriculo-ventricular wall.

The aorta and left ventricle are slit open in a similar manner by passing a blade of the scissors into the aorta, past the semilunar valves, and into the ventricle. The auriculo-ventricular wall is finally divided through the mitral opening. By these incisions the valves and different portions of the heart cavity are successively exposed for examination. The coronary arteries should also be slit open and inspected.

The water test for the competency of the semilunar valves is carried out by pouring water into the artery and observing the result, interfering blood clots being removed. For the auriculo-ventricular valves water is forced into the unopened ventricle with a syringe.

The *lungs* after removal are cut open by a long longitudinal incision, and any specially diseased portions further examined by secondary incisions.

The *abdominal viscera* after general exploration *in situ* may be removed and examined in such order as may be most convenient.

The *spleen* is easily removed by drawing it into view and dividing its attachments; it is then slit open and its interior examined.

The *stomach and intestines* are first examined from the exterior throughout their length, *in situ*. For further examination the stomach and intestines may be removed all together, or separately, or in any portion desirable. If biliary or pancreatic lesions are present the duodenum should be left *in situ* and cut open to expose the outlet of the common duct, which is then slit open. To remove the intestine, tie two ligatures around the lower part of the sigmoid flexure and cut the bowel between them. Then, by dividing the mesenteric attachment close to the bowel, the intestine is freed from below upward, being placed in a basin as removed. The bowel may be again tied off at the duodenum, or the duodenum and stomach may be removed with it. The stomach may be removed alone by ligating at both ends (especially if its contents are to be examined for poison), and severing its attachments. The intestine and stomach may be slit open their entire length at the sink, with the enterotome, washed, and examined.

The *pancreas, biliary organs, and liver* may be examined *in situ* or after removal.

The *kidneys* are enucleated with the fingers after incising the

overlying peritoneum, brought into view, and completely freed by dividing the renal vessels and ureters, after examining these. The kidney is cut open by a longitudinal incision along the convex border of the organ, extending into the renal sinus and dividing the organ into lateral halves.

The *bladder* may be opened *in situ*; or for more thorough examination of it, the prostate, and urethra, its attachments may be divided from within the abdomen down to the perineum, and the penis and urethra dissected out, from the outside, to the perineum. The attachments at the base of the bladder are lastly divided, and the urethra is drawn into the abdominal cavity.

The *female genitals* are easily removed for examination.

Examination of the Brain.—To obtain the brain a transverse incision through the scalp is made over the vertex of the skull, connecting the upper ends of the two ears. As few of the hairs should be cut off as possible; and to avoid this altogether the knife is best introduced with its blade outward and its back against the skull, and in this position swept along the line of the incision. The incision should go to the bone, and divides the scalp into an anterior and a posterior half or flap. The anterior flap is then dissected loose forward, being turned wrong-side out and drawn down over the face as it is loosened; the posterior flap is loosened and drawn backward in the same fashion. In dissecting off the flaps the tissues should all be removed to the bone, including the temporal muscles laterally. The entire top of the skull being thus laid bare, the bone is sawed through entirely around the skull in a horizontal plane, from the superciliary ridge to the occiput. It is best to saw through the outer table and break the inner table with a chisel, to avoid injuring the brain. The bone being entirely severed, by means of a hook inserted under the frontal edge the entire calvarium can usually be easily pulled off without injury to the brain, though sometimes firm adhesions of the dura mater prevent this. The brain is loosened from its attachments to the base of the skull, and the spinal cord is severed with a long, narrow knife as far in the spinal canal as possible. The brain, thus freed, is examined externally by inspection and palpation; it is then cut open in order to expose its interior.

A number of methods of sectioning the brain are in use, and the procedure may be varied according to circumstances. One

method consists in laying the brain with its base downward, and with a long, broad, and thin-bladed knife, kept moistened with water, cutting the entire organ into a succession of thin horizontal slices from above downward. Each half of the brain is sliced separately, the incisions beginning at each side and being carried inward nearly but not quite to the middle line, so that all the sections are left attached at the middle like the leaves of a book.

Examination of the Spinal Cord.—To remove the spinal cord the body is turned face downward, the spinal column is exposed, and the laminae of the vertebrae are divided on each side with a saw having a rounded end, with a double saw, or with bone forceps. On removal of the tissues thus freed, the cord is exposed and may be dissected out.

In the course of the examination of the various organs, their weight and measurements are noted, and such special observations made as the circumstances may suggest. There are many minor features and structures in various parts of the body to be considered and looked into, as the condition of the various arteries, the lymphatic glands, etc., that cannot be mentioned in detail but will naturally occur to the operator. Portions of tissues are set aside for microscopical examination as required, and bacterial investigations may be pursued.

Departures from the regular routine are sometimes necessitated by the exigencies of the case, as when a complete examination is not practicable, or when the autopsy is performed in a private house and without the facilities of a morgue. The operator must in such cases conform to circumstances, and do his best within the limitations fixed. Thus, much can be done by simply reopening a recent operation wound (abdominal, for instance); or with only the abdomen opened the thoracic organs can be removed through the diaphragm.

Bacteriological examination of certain organs and fluids is frequently required, as of the liver, spleen, kidneys, lungs, blood of the heart, and any specially affected areas. This may be done by cover-glass preparations and by cultures.

Cover-glass preparations are made by spreading a drop of pus, exudate, blood, etc., on a cover-glass with a platinum loop, or between two cover-glasses pressed together; or by rubbing the fresh and moist cut surface of the tissue involved over the cover-

glass so as to leave a thin film or "smear." These cover-glass spreads are dried, fixed, and stained in the usual way.

Cultures are made with pains to avoid contamination. For this purpose, in making cultures from solid organs, the surface is first scorched with a hot spatula, and through this burned area a puncture is made into the organ with the heated spatula; a strong platinum wire or loop, previously flamed, is passed into the tissues in the puncture thus made, and culture tubes are inoculated with the material obtained.

Cultures from the blood of the heart are made before removal of the organ from the body; the surface of the right auricle or ventricle is scorched with the hot spatula; through this area with a sterile knife a puncture is made, through which a drop of blood is obtained with a platinum loop for inoculation on the culture media. Cultures from pus, exudates, exposed surfaces, etc., are to be made without contamination by the hands or instruments.

The best culture medium is blood serum. The platinum wire, charged with the material to be inoculated, is drawn or rubbed over the surface of the coagulated serum. Ordinarily this will scatter the germs sufficiently to produce discrete colonies; but with pus or material very rich in bacteria it may be advisable after inoculating one tube to draw a sterilized platinum wire over the surface of this first tube and with the germs thus diluted to inoculate a second tube. Agar may also be used, either in slants or Petri plates. If required, animals may be inoculated directly from autopsy material.

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